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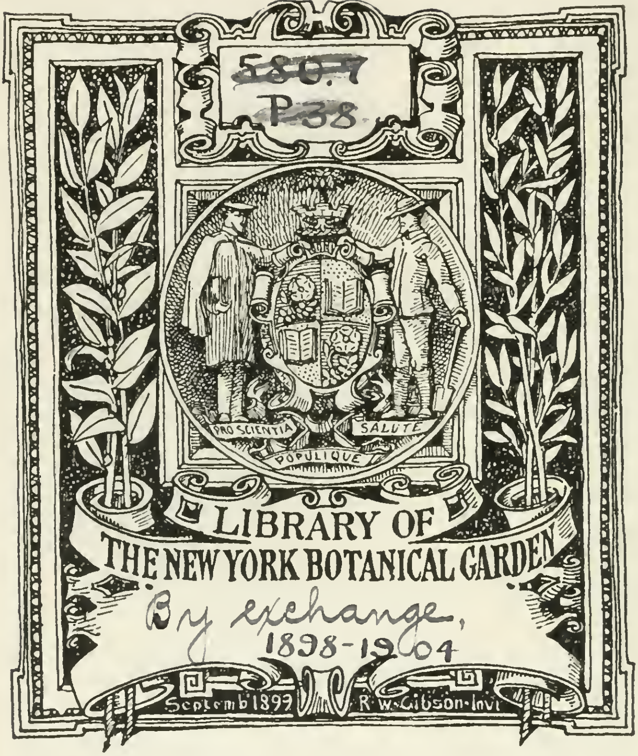
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Observations on Conopholis Americana.

(*WITH PLATES I-VI.*)

By Lucy L. W. Wilson, Ph. D.

Head of the Biological Department, Philadelphia Normal School.

[Thesis presented to the University of Pennsylvania]

I. HISTORY AND LITERATURE.

IT is now nearly twenty-two hundred years since Theophrastus Eresius (died 286 B. C.) appropriately bestowed upon a plant, which still bears the same name, the title of *Orobanche*, literally peachoker. This name in extended form was afterwards applied to the whole order, which now includes some hundred and fifty species. Yet, in spite of the lapse in time, the family itself has been comparatively little studied. This seems doubly strange when one recalls their peculiar habits of growth, habits so well known and so characteristic that it was in describing them that Micheli in 1720 ("*De Orobanche*") first used the now common word parasite.

The literature cited in Engler-Prantl's "Pflanzenfamilien" by Günther Beck von Mannagetta in 1891 is given below.*

To this should be added Chatin's "Anatomie Comparée des Végétaux. Plantes Parasites," (Paris, 1892).

The absence of literature is especially noticeable in the case of the species which is the subject of this paper. With the exception of the mention of its gross specific characters in the various Floras and Prodromuses, most of which have been cited above, from the time of Linneus to the present, nothing had been added to our knowledge of the plant, except what was implied in Wallroth's separation of it from the genus *Orobanche*, where it had been placed by Linneus, and its erection into a separate genus *Conopholis*, in 1825. Of this genus it was the solitary species until Sereno Watson described a second, *Conopholis mexicana.*

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* Wallroth, Orobanches Generis (1825); Vaucher, Monographie des Orobanches (1827); G. Beck, Monographie der Gattung *Orobanche* (1890), Bibl. Bot. Heft 19; Baillon, Hist. des Plantes (1891); Endlicher, Gen. Plant (1836–1840); Reuter in D. C. Prod. XI (1847); Hooker and Bentham's Gen. Plant. II (1873); Hooker, Flora Brit. Ind. (1885); Gray, Syn. Flora N. Am., II (1886); L. Koch, Die Entwicklungsgeschichte der Orobanchen (Heidelberg, 1887); Hovelacque, Rech. sur l'appareil veget. des Bign. Rhin. Orob. (Paris, 1888).

In 1892, it is true, Dr. Chatin published his work on Parasites, in which three pages of text and one of illustrations lithographed from free-hand drawings, are devoted to the histology of *C. americana.* The material with which he worked, however, must have been quite scanty and young, for a careful study of the fine anatomy of the plant shows that in most instances his statements and drawings do not conform to conditions in the adult plant. The details of the many points of difference will be given later on. Material for the present study was collected by Professor Macfarlane in the Allegheny Mountains, near Gallitzen, during June of 1896, and my investigations have been made under his direction.

II. GEOGRAPHICAL DISTRIBUTION.

Conopholis americana, as its specific name indicates, is a distinctly American plant. It is not very commonly found, but has rather a wide distribution. Gray says in the last edition of the "Manual," "New England and Michigan, south to Florida and Tennessee, May and June."

The following more exact information is due to the kindness of several botanists to whom I desire here to make acknowledgment.

New York.—Staten Island, Garretson's. A. A. Tyler (Herbarium of Lafayette College, Dr. Thomas C. Porter).

New Jersey.—Sussex County, near Newton. A. P. Garber (Herbarium of Lafayette College, Dr. Thomas C. Porter).

Pennsylvania.—Chester County, R. Kipington. Lancaster County, Mouth of Tuequanhe, N. A. Heller. Franklin County, Mercersburgh. Allegheny County, Wall's R. R., S. W. Knipe. Mercer County, Middlesex, A. P. Garber.

All of the above information was gained from the Herbarium of Lafayette College and given me by Dr. Thomas C. Porter. It is also reported from Delaware County (Dr. George Smith's Catalogue), but no locality is given. Bucks County (J. S. Mayer's Catalogue). Chester County, rich woods along the Brandywine (Darlington's *Flora Cestrica*).

Virginia.—Blue Ridge Mountain, Turhe's Gap, Albemarle County, J. D. Tinsley. Smyth County, J. K. Small (Herbarium of Lafayette College, Dr. Thomas C. Porter).

West Virginia.—Cheat Mountains, L. C. Corbett. Decker Creek, L. C. Corbett.

North Carolina.—Watauga County, Blowing Rock, Small and Heller (Herbarium of Lafayette College, Dr. Thomas C. Porter).

South Carolina.—Northern part, above lower limits of Piedmont plateau, W. W. Ashe.

Georgia.—Northern part, W. W. Ashe.

Florida.—Near Apalachicola. This region has now been built up, and the plant is in consequence no longer found there; A. W. Chapman. Chattahoochee, A. W. Chapman. Lake City, P. H. Rolfs. Ocala, J. D. Smith (Baltimore). Hibernia, W. M. Canby (Herbarium of Lafayette College, Dr. Thomas C. Porter).

Ohio.—One and a half miles east of Oberlin, Albert Norris.

Indiana.—Locality not given, Thomas MacBride.

III. Time of Flowering.

The flowering shoots, which are the only parts of the plant that appear above ground, have been found in the Pennsylvania woods in early May, just beginning to flower (May 10, 1890, Lancaster County, N. A. Heller) and continue in full bloom until certainly the third week in June (Gallitzen, Pa., Professor Macfarlane).

The following dates have been variously reported:

Chester County, June 27, past flowering.

Franklin County, May 23, in flower.
Allegheny County, June 19, in full flower.
Mercer County, June 12, in full flower.

It was reported from Staten Island, N. Y., as in fruit July 11, 1895; and coming southward into southwestern Virginia, it was there already past bloom as early as June 8, 1892; in flower, but passing out, in Blowing Rock, N. C. (exceptionally high), June 16–17, 1892; while in Florida, it was in flower as early as March and April.

Apparently, then, the period of flowering lasts about two months, beginning in early May in our Middle States, but, as might be expected, even two months earlier farther south.

IV. Relationship to Other Members of the Order.

The flowering stalks, always some distance from the trunk of the host, are remarkable in appearance. Unlike the other members of this order, they are thick and fleshy. Their color is a chestnut brown. They are six inches in length when fully grown, covered with thick, membranous-fleshy scales, in the axils of the upper ones of which are found the flowers. These are of the same color as the leaves, and like all of the *Orobancheæ*, have a marked resemblance to non-parasitic *Scrophulariaceæ*. There are two bracts at the base of the calyx, which is irregularly four to five-toothed and split posteriorly to the base. The upper lip of the two-parted corolla is entire, or notched; the lower three-parted with the middle division obtuse and larger than the other three. The stamens are protruded, and the one-celled ovary is full of ovules attached to four parietal placentæ. These differ but slightly from the flowers of the other closely related American genera, *Phelipæa* and *Aphyllon*. The most noticeable point of difference is the protruded stamens.

The calyx of *Phelipæa* is not cleft down the middle, and there is a well-developed ovarian gland, of which there is also a microscopic indication in the very young flowers of *Conopho-*

lis. It has also the two bracts. According to Chatin, there is a close correspondence in the peculiar histological characteristics of the two genera. He figures for both the same arrangement of bundles, and the same kinds and distribution of parenchyma.

This seems to indicate that these two genera certainly, and possibly *Aphyllon* also, might be better included as species of a single genus. Unfortunately, as I shall show later, no reliance can be placed on Chatin's work.

V. The Host Plant.

The host of *C. americana* is given by Gray as the oak. Dr. Thomas Porter gives the same observation, as do the following: P. H. Rolfs, L. C. Corbett and W. W. Ashe. Mr. Albert Norris reports to have seen it in two cases under maple trees, and Rolfs reports it under oaks and beeches. In all cases, where the parasite has been traced to its attachment, it has been invariably found to be parasitic on the oak. It is, however, frequently quite a distance from the trunk of its host, which may account for its being found under other trees. There is no positive evidence in favor of any other than an oak attachment.

VI. Duration of Life.

The flowering shoots are undoubtedly of annual development, probably from buds formed the previous year in tubercles whose length of life must depend upon the capacity of the host to feed the parasite.

It was impossible to determine accurately the age of the tubercle. The relationship between its size and the age of the oak root was the only clue that I could get of the age of the parasite. Tubercles half an inch in diameter, with small buds which had apparently just pierced the cortex, were found on roots three and four years old. The oak root, whose photograph is shown in Plates I and II, is fully eleven years of age. The tubercle itself measured about six inches in diameter.

From these facts and others which are tabulated below, I concluded that the seed usually germinated on quite young roots; but that it did not send up a flowering shoot until perhaps its fourth or fifth year of existence.

The subjoined table shows the size of some tubercles, and the corresponding age of the oak root on which they were parasitic:

Age of Oak Root in years.	Diameter of tubercle.
3	1 inch.
5	2 inches.
5	3 inches.
5	3½ inches.
6	1¾ in. x 1 in.
6	2½ inches.
6	3½ inches.
7	2 inches.
7	2½ inches.
8	2½ inches.
8	3 inches.
9	4 inches.
10	4 inches.
11	6 inches.

VII. RELATIONSHIP BETWEEN CONOPHOLIS AND ITS HOST.

The flowering shoots of *C. americana* are found above ground usually some distance from the oak and often forming almost a semi-circle of growth around it. Sometimes above ground, too, are found the tubercles from which the stalks proceed. This was particularly true at Gallitzen, Pa., as observed by Professor Macfarlane. But it is only by digging below the ground that any idea can be had of the huge excrescences made by the parasite and the roots of the oak. Three of these are figured on Plate I, which is a photograph of a small portion of the underground material dug up at Gallitzen, Pa., June, 1896. Younger stages are drawn on Plate VI, Figs. 1, 2, 3 and 4.

It will be noticed that from all of these tubercles spring flower stalks in various stages of development. The larger tubercles, such as are figured in Plate I, are almost completely covered with such stalks. Curiously enough, however, there are few transition stages between the adult stalks and the very numerous young buds. The material was gathered near the close of the flowering period. Therefore, it may be that these buds remain dormant until the next year. It seems quite as probable however, that, like other parasites, they are capable of very rapid growth, and that transition stages are rarely found because the transformation takes place rapidly.

The buds are protected by the scale leaves, which, in the young state, are rather fleshy than membranous. The tubercle itself is covered with a thick, coarse, porous, dark-brown "bark," which scarcely holds together the innumerable granules of sclerenchyma which make up the great mass of the excrescence. Plates II and III, which are photographs of a tubercle and the host oak root cut through the middle, show quite clearly the enormous quantity of sclerenchyma in such a growth. Plate V, Fig. 5, is a drawing of a cross section through one of these patches. It will be noticed that it resembles markedly sclerenchyma groups, which are normally developed in the cortex of the oak, and are, indeed, characteristic of it.

These photographs do not show what was particularly striking in the material itself, namely, that wherever a flower stalk had been, there were left behind large masses of sclerenchyma embedded in dead cellular tissue.

Plates II and III alike show three large nodules, the centres of which are undoubtedly oak, much more compact and with fewer groups of sclerenchyma than the others. The lower nodule of Plate II indicates, perhaps, how the oak wood gradually became isolated from the surrounding wood, thus forming apparently comparatively unattached centres. The middle one of these three large nodules dropped from the rest, plainly showing that it now had no organic connection with the rest of the oak, and this in spite of the fact that there can be no doubt that its centre was once a part of the oak root.

It will be seen (Plate III) that these patches of sclerenchyma are found in the cortex of the oak root, that they gradually become larger and more numerous until finally they make up the bulk of the excrescence, and also that the diameter of the root increases in proportion to the amount of sclerenchyma present.

Not only is the sclerenchyma continuous, but the "bark," it will be noticed, covers, without apparent interruption, the root of the oak and the tubercles.

The lines of invasion of the host by the parasite may be made out clearly in both of these plates, particularly if one looks carefully at the lower half. But in such natural specimens it would be difficult to understand the relationship of the parasite to the host, even with the most careful microscopic study. A study of the younger nodules, however, enables us to understand the larger growths.

The youngest tubercle which I was able to find in the keg of material brought from Gallitzen was about half an inch in diameter, and growing at the end of a root fully three years old. The tips were already developed, tiny scales covering the rudiment of a flower stalk (Plate VI, Fig. 1). Figs. 2 and 3 are drawings of tubercles of about the same size and apparent age. Fig. 2 has, however, more flower-stalk buds, which are farther developed in Fig. 3. In Fig. 2 is drawn a tubercle, beyond which the root still extends. The same thing will be noticed in Plate I. Nevertheless it was fairly unusual to find roots extending beyond even the younger tubercles. Like other parasites, it seems most frequently to prevent their growth and development, by cutting off and absorbing the nourishment originally intended for the younger parts of the root.

Like the older tubercles, these were masses of sclerenchyma, covered with bark and held together by soft delicate tissue.

It was very difficult to get vertical sections of any of these tubercles, owing to the large quantity of sclerenchyma, the softness of the connecting tissue, coupled with the very different character of the oak root. Still the photomicrograph reproduced in Plate V, Fig. 5, gives a very fair idea of the state of affairs, although it is not quite through the middle of the tubercle, and does not, in consequence, show at its apex the scale leaves which cover over and form a part of the very young flower bud, which was noticed in Plate VI, Fig. 1.

The "bark" is very thick and clearly made up of several layers. Below it are patches of sclerenchyma that lie embedded in soft, rather long-celled tissue, which, both in the photograph and in reality, takes the form of anastomosing lines. Completely surrounding the patches of sclerenchyma, at the base of the flower stalk and in it, this becomes gradually transformed into vascular and parenchymatous tissue.

The apparent line of demarcation between this tissue and that of the oak host is plainly shown in the photograph. It extends longitudinally across, with downward invasions, into the solid oak below, which, in its turn, spreads out fan-wise above.

At first sight it would seem that this strongly marked line was the separation between parasite and host; that all above it was certainly *C. americana*, and all below was equally certainly *Quercus rubra*. There are, however, one or two objections to this:

The sclerenchyma patches strikingly resemble such as the oak normally develops, in a more limited quantity and at a later period, in the cortex tissue. At the same time the ramifying soft tissue is plainly and unmistakably that which afterward becomes differentiated into the fundamental and the vascular tissue of the parasite, so that above this line of apparent separation there is a possibility that both host and parasite are present. Now, it is perfectly conceivable that the irritation to the oak caused by the continued downward growth of the parasite would have the effect of hastening the formation of the sclerenchyma, thus effectually resisting further attack of the parasite, which would then perforce penetrate more and more deeply into the oak with the same result of sclerenchyma formation following its progress.

In this connection, the drawings in Plate VI, Figs. 1 and 2, are most suggestive. The root of the oak actually rises up to meet the parasite. In the vertical section (Plate V, Fig. 5), its solid tissue spreads outward and upwards into the softer tissue above.

Plates I, II and III can now be understood. Each of the three tubercles in Plate I is the growth of a single seedling. The difference in size is the consequence of the varying age of the parasitic mass. These tubercles are partly the result of the growth of the host, due to irritation of its tissue. This growth of the host is shown in the swollen appearance of the root, just below as well as in the base of the tubercle, and also in the masses of sclerenchyma which make up the greater part of each tubercle. The parasite proper consists of the flowering stalks and buds, also of an undetermined part, perhaps the whole of the parenchymatous tissue, which ramifies through the tubercle in every direction, surrounding and holding together the sclerenchyma patches and, as is shown in Plates II and III, penetrating the as yet unchanged bast and wood of the oak root. To the host, on the contrary, besides its evident possessions, may be attributed the "bark," the sclerenchyma, and, possibly, a part of the ramifying parenchymatous tissue.

The only argument against this is the fact easily seen in Plate IV, Figs. 1, 2, 3 and 4, that the patches of sclerenchyma are found at the base of flower shoots, in what is undoubtedly the tissue of the parasite. On the contrary, however, they are never found, except at the very base of the adult shoot.

The tubercles are, in the main, a modification of the host, within which develop endogenously the buds of the flower shoots, which then break through the thick, protecting "bark."

This peculiar relationship of parasite and host recalls in its development and growth the *Balanophoreæ*, or even the *Rafflesiaceæ*, rather than the typical *Orobancheæ*, whose connection with the host plant in all genera and species which have been carefully worked out, is a much more evident and a much less intimate one. These always develop some earth roots in addition to the haustoria. This is never true of either of the above orders, in both of which the flower is the only part rising free from the host.

Langsdorffia and *Balanophora* are typical members of the order *Balanophoreæ*, whose history and parasitic relationship have been carefully worked out by Sachs and Eichler, more particularly the latter. The seed of each, in germinating, destroys the bark and cortex of the host roots, lays open, lacerates, and unravels the tissues in the search for food. Then the woody bundles of the host ascend into the substance of the parasite, spread out like a fan, and become so interlaced with the cells and vessels of the parasite that it is quite impossible to distinguish one from the other.

VIII. Histology of C. americana.

Under this head will be taken up mainly the microscopic anatomy of the flowering stalk. As much of the histology of the tubercle as I was able to make out has been already discussed. But to make what follows clearer, I will recapitulate the chief points mentioned. All are quite plainly demonstrated in Plate V, Fig. 5, namely, the thick "bark," continuous and identical with that of the oak, the masses of sclerenchyma, and the threads of soft parenchymatous tissue surrounding these masses.

MATURE FLOWERING SHOOTS.

A cross section, through any part of the flowering shoot above the tubercle, shows the following structure:

(*a*) An epidermis made up of rather thick-walled cells, filled with a yellowish-brown protoplasm.

(*b*) Parenchyma, made up of cells of varying size and thickness. The intercellular spaces are large and frequent.

(*c*) Two concentric rows of separated collateral fibro-vascular bundles.

All of these points may be seen in Plate V, Figs. 1 and 3, and are not particularly noteworthy, although the double row of separate bundles is rather uncommon.

Figures 2 and 3 on Plate V show the very remarkable relation of these bundles. Each bundle of the inner row has internally xylem, made up of xylem cells and well-developed spiral tracheæ. Next to the xylem is found the phloem, which a longitudinal section proves to consist of both sieve tubes and companion cells. Adjacent to the phloem are a number of parenchyma cells, whose walls are so angular and so much thickened, that in the photograph, these bundles appear to be bi-collateral. That such is not the case, however, is easily proved on longitudinal section, when the parenchymatous nature of these cells is at once visible. Even in cross section, the color of the walls differentiates the wood from the thickened parenchyma.

The bundles of the exterior row have the same structure as those of the interior, only the xylem is now exterior so that the phloem masses of the two rows face each other.

Plate V, Fig. 4, shows a photomicrograph of one of the exterior bundles. The wood is shown above, then comes the phloem, flanked below by the thickened parenchyma. On either side are the parenchyma cells characteristic of the major part of the flower stalk. The intercellular spaces are quite noticeable.

Cross sections of the stalk, which happen to be under or at the base of the leaf (Plate V, Fig. 3), show invariably a migration of bundles from the inner toward the outer row, and from the outer toward the leaf. This would seem to indicate that both rows of bundles consist of leaf traces, but for reasons which will be given later, it is certain that this is only directly true of the outer row, which may be considered, then, to be both cauline and leaf trace, whereas the inner row is cauline.

The epidermal cells of the flower stalk are somewhat irregularly thickened, and contain stomata. Strange to say, there are no stomata on either surface of the leaves, another indication of the greater depth of parasitism to which this particular member of the *Orobancheæ* has descended.

The *Orobancheæ* in general have more numerous stomata than most parasitic plants (Unger, Exantheme d. Pfl.), but *Conopholis* is not the only member in which they are curiously placed. *Lathræa Squamaria* has them on the pistil only (Krause), while in the closely allied species, *L. clandestina,* they are in normal numbers and on the leaves (Duchartre).

IMMATURE FLOWERING STALKS.

Plate IV, Figs. 1, 2, 3 and 4, are photomicrographs of vertical sections through young buds of flowering shoots. It will be observed that in Figs. 1, 2 and 4 are plainly seen the outer and an inner row of fibro-vascular bundles already described in the adult shoot. These anastomose with each other and in no case "end blindly beneath the apex of the stem" as they are said to do in other genera of *Orobancheæ* (de Bary).

Figure 3 is taken from a section cut at such an angle that the anastomosis of the numerous bundles of both the inner and outer circle is demonstrated.

In Fig. 2 it will be seen that the outer circle consists of leaf-trace bundles, and also that the inner circle anastomoses with it at intervals below, as well as at the apex. This as well as Fig. 4 showed by higher magnification the spiral tracheæ, which make up about half of the fibro-vascular bundles. The dark spots at the base of all these figures are the sclerenchyma masses, which just below these buds make up almost the entire mass of the tubercle.

Each cell of the parenchyma of the shoot, in addition to a well-defined nucleus and nucleolus, contains from one to seven clear spherical, highly refractive bodies, on which even hydrochloric acid made no visible impression. Nevertheless, a set of sections left over night in the acid had in the morning scarcely a granule left, while another set which remained in alcohol over night still retained them.

These bodies are most numerous in the apex of the stalk. Further down they have lost their spherical outline, look to be disintegrating and finally disappear. About the region where they begin to disappear the patches of sclerenchyma begin to appear.

The bundles are well differentiated almost to the apex. They are made up, in about equal halves, of xylem and phloem, the former consisting of spiral tracheæ and wood cells. In the adult phloem a few sieve tubes are found. The relatively large amount of phloem is interesting and characteristic of parasites in general. For obvious reasons such a plant does not need much wood, and the rather large amount in this case has some relation to the fairly abundant stomata, which are so often lacking in other plants of a similar habit.

Cross sections of a very young rhizome, just before it leaves the tubercle, seem to indicate that the outer row of bundles is first formed and that the inner circle is developed slightly later. Nevertheless, since the inner row represents the normal group of bundles, this is so contrary to what might be expected that my evidence seems to me scarcely to justify more than the suggestion that the order of development may be as indicated.

Chatin figures a third row of bundles—leaf-trace bundles he calls them—in the angles of the rhizome and flower stalks, quite close to the epidermis. In many thousands of cross section of all ages and from all regions I have been unable to find anything of the kind. As I have already proved, the outer row of bundles is both trace and cauline, while the inner is exclusively cauline. Bundles of the inner group occasionally anastomose with those of the outer, however, and may in that way indirectly reach the leaves, although even this seems improbable.

Cross sections of the rhizome, made at its base, show a large number of bundles, rather irregularly arranged, it is true, but still plainly referable to two rings.

Chatin's statement that there are three concentric rings of bundles, together with the drawing of the same, can only be understood on the supposition that his material consisted of very young shoots, of which he made but a single section.

IX. Leaves.

The leaves of *C. americana* are numerous and imbricated. To their peculiar appearance, indeed, is due the generic name, *Conopholis*, *i. e.*, cone-scale.

As has been already stated, they are yellowish-brown in color, at first inclined to be fleshy, but afterward membranous in texture.

The epidermis consists of thick walled cells, and is much better developed on the under than on the upper surface. Plate VI, Fig. 6, shows the bead-like thickening of the walls in the under epidermis. To this, perhaps, as Chatin suggests, is due the absence of stomata on the leaf, and their presence in the thinner-walled epidermis of the flower stalk.

The walls of the parenchyma tissue immediately within the lower epidermis are much thicker than those of the same cells under the upper epidermis. There is no indication of palisade cells.

The mesophyll resembles greatly, both in the shape and size of its cells and of the intercellular spaces, the parenchyma of the leaf stalk. In some of the cells are leucoplasts, but they are erratically distributed and not numerous. The clear refractive bodies, probably a glucoside, mentioned as existing in the cells of the young flowering shoots, but absent from the adult stalk, are here very abundant.

The bundles are collateral, as in the flowering shoot, lie parallel to each other, and vary in number from seven to eleven. Usually three of them are larger than the others.

X. FLOWERS.

The description of the flower has been already given, and allusion made to a rudimentary ovarian gland seen in a cross section of a young flower bud. This ovarian gland is particularly interesting in view of the fact that a well developed one is found in the adult flowers of *Phelipæa*, which in this and many other respects, already noted, closely correspond with those of *Conopholis.*

The fruit of *C. americana* is a two-valved, single-celled capsule. On the middle of each valve are developed two parietal placentæ bearing numerous seeds of fair size.

The seeds have well-developed endosperm, with small undifferentiated embryos.

A detailed study of the floral structure and of the embryology will be given in a later paper.

The following is a brief summary of results:

1. *Conopholis* is parasitic on the oak, and may form a fringe of growth round the trunk, at a distance of ten or more feet.

2. It is perennial to the extent of at least eight to ten years.

3. It first affects young roots, and usually starves the portion beyond the point of infection.

4. The union between parasite and host is an extremely intimate one, the parasite being practically developed endogenously within its host, which rises up and encloses it after its

germination. The resemblance in this respect is not to members of the *Orobancheæ*, but exactly to the *Balanophoreæ* and *Rafflesiaceæ*.

5. The irritant action of the parasite causes swelling up of the host root, and enormous multiplication of its sclerenchyma patches.

6. Each parasitic "tubercle" consists of a bark, sclerenchyma masses and possibly some cellular tissue belonging to the host, and of cellular tissue and bundle issue, chiefly developed in the flower stalks of the parasite.

7. The flowering shoots show two concentric rows of bundles.

8. The phloem masses of the bundles face each other.

9. Stomata are present over the flowering shoots, but absent from the leaves.

10. The leaves are brownish-leathery when mature, and are devoid of palisade tissue.

11. In cells of the leaves and young flowering shoots are numerous clear refractive bodies which may be of a glucoside character.

12. The flowers show a small ovarian nectar gland.

EXPLANATION OF PLATES I-VI.

Plate I. Three growths of *Conopholis*, on oak root, one-half natural size.

Plate II. Longitudinal section of *Conopholis* and oak root.

Plate III. Longitudinal section of *Conopholis* and oak root, opposite half to that figured in Plate II.

Plate IV, Figs. 1–4. Longitudinal sections of young flower shoots on oak tissue.

Plate V, Figs. 1 and 3. Transverse section of flower shoot of *Conopholis*, Fig. 1 × 30°, Fig. 3 × 50°. Fig. 2. Portion of stem showing double circle of vascular bundles, × 75°. Fig. 4. Single bundle, × 350°. Fig. 5. Longitudinal section of flower bud from young plant of *Conopholis*.

Plate VI, Figs. 1–4. Young plants of *Conopholis* attached to oak roots, natural size. Fig. 5. Patch of sclerenchyma cells from parasitic swelling. Fig. 6. Cells from epidermis of leaf of *Conopholis*.

Recent Observations on Amphicarpæa Monoica.

By Adeline Frances Schively, Ph. D.

Honorary Fellow in Botany.

THE results of certain experiments had not been determined, when my paper published in the last number of the "Botanical Contributions of the University of Pennsylvania" went to press. In order that the observations now to be discussed may be clearly presented, certain allusions to statements in the paper mentioned above will be found necessary.

A. monoica bears above ground, during August, racemes of purple flowers, whose productiveness is quite variable; but in most seasons a fair number of legumes may be gathered. These legumes are lanceolate or falcate in shape, and contain rarely two, usually three, seeds, which when ripe are grayish-green, flecked with purple. When immature, the legumes are green; later they become brown, and dehisce in the usual manner. The dorsal and ventral sutures are quite prominent, and are markedly hairy. The walls of the legume are not in close contact with the seeds; upon the outer surface of the walls a few scattered hairs occur.

In September, aerial greenish cleistogamic flowers appear, and by October have produced legumes differing in shape from those already described; the number of seeds varies from one to three; but the color of the seeds and the general features of the legume are similar to those resulting from purple flowers.

During the entire season, subterranean flowers are constantly developing. The legumes here produced are pyriform, and typically contain but one seed, which occupies the entire space. When immature, the legume walls are white, or a very pale purple; when mature, the color varies from rich pink purple to purplish-brown. The seed has a whitish coat, upon which irregular purple patches occur.

The histological structure of these legumes and their seeds presents striking characteristics. These will now be discussed under the proper headings.

Legumes of Aerial Type.

The outer epidermal cells are irregularly isodiametric; stomata are very numerous. The few hairs that occur are of two varieties—small bladder-like forms and long unicellular ones. A cross section shows that the layer of epidermal cells is quite shallow; the hypodermal layer consists of indurated tissue. Maceration, and also surface view, shows this layer to be made up of rods, varying in length from .2 to .5 of a millimetre. They are pointed at each extremity and fit closely together. In the cross section from eight to ten rows of parenchymatous cells are next seen; occurring at irregular intervals are the vascular areas, accompanied by tannin canals. The inner epidermis has become quite indurated; in surface view the cells appear to have fused into long, narrow cells resembling fibres; these structures run longitudinally in the legume. The mesophyll is richly supplied with chloroplasts. The vascular areas, which constitute the dorsal and ventral sutures, show long delicate fibres, not easily separable, even after prolonged maceration. From examination of alcoholic and dry material at hand, dehiscence seems to be the result of increased induration of the inner epidermis and some cells of parenchyma adjacent to it.

Seeds of Aerial Type.

Sections of the seed-coat show that the epidermal layer, which consists of indurated cells, occupies about one-third of the entire thickness of the coat. These cells contain the purple coloring matter—anthocyanin—apparently quite evenly distributed, and merge into a narrow, homogeneous, colorless band, which may properly be termed the cuticle. The hypodermal cells are curiously shaped, their walls are exceedingly thickened, possessing flanges which abut upon each other. Then follows a layer about eight cells deep, mainly of thin-walled tissue. The inner cells, bordering directly upon the cotyledons, are flattened and thick-walled. Marked increase is noticed in the depth of the epidermal and hypodermal layers, and in the extent of induration possessed by all cells if sections are made in the neighborhood of the hilum.

The external epidermis of each cotyledon consists of two rows; these cells are smaller than those in the remaining portion of the cotyledon. The latter cells measure .075 of a millimetre. Starch and protein granules are present in all cells, excepting those which have been termed epidermal; in these protein alone may be found. The starch granules are spherical, measure .0075 of a millimetre, and have no characteristic markings. The protein granules are so minute that nothing satisfactory concerning their structure has been determined.

LEGUMES OF SUBTERRANEAN TYPE.

The epidermal cells of the subterranean legume resemble in shape those described for the aerial, but the stomata are situated at the apices of small papillæ. In size and number there is little difference from those of the aerial legume. The entire surface is covered by a dense growth of hairs—very many of the small bladder-like form, long unicellular ones, and fewer of a kind somewhat resembling the last named, but differing from them in developing from a multicellular base, that is raised conspicuously above the surrounding surface. A cross section of the legume is remarkable in the absence of all indurated tissue. Delicate fibres belonging to the vascular areas are, of course, present; but the inner epidermis has not changed its thin-walled character, nor does the outer hypodermal region show any indications of induration. As the sutures are not very prominent, the strong vascular bundles are not readily discernible. Anthocyanin is irregularly diffused through the outer parenchymatous cells. The purple hue deepens as maturity approaches. The average number of mesophyll cells is six, but occasionally eight are found. The plastids seen here are of the same size as the chloroplastids observed in the aerial legume, but one or two only are found in a cell of the subterranean, ten or twelve in a cell of the aerial legume. The legume walls become brown after they remain under ground for some time. I think this must be partly due to excess of water supply, for legumes which I have kept in slightly moistened earth retained the purple color for some weeks. Subterranean legumes never dehisce.

Subterranean Seeds.

The seed-coat exhibits an epidermal structure which both in form and possession of color closely resembles the conditions described for the aerial seed. Excepting this, no other indurated tissue is to be observed in the subterranean seed coat. The cells of the cotyledons measure .15 of a millimetre. The arrangement and contents of the epidermal cells are similar to those in the aerial seed; these cells too are smaller than those in the remaining portion of the cotyledons. The starch granules are ellipsoidal and measure .015 of a millimetre. Protein granules are more numerous in the older seed than in the younger specimens; in the latter, starch is comparatively more abundant. It is suspected that the same condition of affairs may be found by comparing aerial seeds of different ages, but unfortunately no material in young condition was at hand. If these facts should prove true, we have evidence of the accumulation of the carbohydrate earlier than that of the nitrogenous compounds.

Experiments.

It had been discovered that the green aerial legumes resulting from the aerial cleistogamic flowers might, by being buried in soil, be converted into others of a purple color and swollen appearance because of great increase in seed growth—in other words, into subterranean pods. There was certainly great similarity between those artificially produced and the ones normally resulting. Specimens of aerial legumes in different stages of development were selected for experiment, as follows: (*a*) with the ovary just emerging from the calyx, (*b*) the well-formed but young legume, and (*c*) older specimens still quite flattened. Satisfactory results were obtained from burying in the soil for a period of from four to six weeks.

Considering the success of the preceding work, it was regarded as possible to obtain a subterranean legume from a purple flower. Accordingly, experiments to verify this supposition were begun in August, 1897. Tiny racemes just showing faint indications of purple color were buried. Racemes situated high on the plant were purposely selected. In about six weeks, fair-sized legumes were obtained—perfect counterparts of those which had been normally produced. Strange to say, too, all were one-seeded. In no case did more than one legume result from a raceme; in some cases none. But in this connection, it must be also remembered, how few legumes often mature from a heavily laden raceme of purple flowers.

The problem now was to ascertain how this treatment had affected the histological peculiarities of the legumes and also the seeds. Specimens resulting from all these experiments were carefully examined and the details in several cases will be given. For reasons which will be made evident in a later part of this paper, the facts gleaned from these will surely serve as strong bases for argument. The series to be explained consisted of legumes resulting from burying purple flowers and from two different stages of legume yielded by the green aerial flowers. They may be referred to as follows:

(a) *Legume resulting from burying purple flower.* (b) *Legume resulting from burying flat but well-formed legume developed from green aerial flower.* (c) *Legume resulting from burying a more mature legume than* (b) *from green aerial flower.*

(a) *Subterranean Legume Resulting from Buried Purple Flower.*

The surface of the legume with its raised stomata, its numerous hairs, including the form with multicellular base, was not to be distinguished from that of any typical subterranean legume. No indurated elements were to be found in the walls. The seed filled the entire cavity; its coat showed but the one indurated layer typical of the subterranean seed; not the slightest indication of the great supporting hypodermal layer of the aerial seed could be discovered. The size of the cotyledonary cells, also the size and shape of the starch granules, agreed with those previously described for the normal subterranean seed.

(b) *Legume Resulting from Burying a Flat but Well-Developed Legume Produced from a Green Aerial Flower.*

The external appearance of the legume was strikingly subterranean in color and fullness, owing to the swelling of the seeds (two in number), which quite filled the space within. The sutures were as distinct as upon any aerial legume. Upon opening the pod, the entire inner epidermis had separated as a sheet of tissue from the remaining portion of the walls. Exactly what agencies caused this is questionable. It may have been the combined action of the new conditions, or perhaps of moisture alone. The surface of the legume was typically aerial in character, possessing no stomatic papillæ, and but a scanty growth of hairs. The short rods in the supporting layer were well developed. Chlorophyll could not be detected, but a pinkish-purple coloring was irregularly diffused through the etiolated cells. The seeds, however, were subterranean in all details of structure and in contents.

(c) *Legume Resulting from Burying a More Mature Legume Produced by Green Aerial Flower.*

This aerial legume having already assumed its characteristic form, showed but little change after experiment. An inclination to turn brown was observed, but this would have occurred under its ordinary conditions. The form of the legume, the size and shape of the seed, and also the histological peculiarities remained unaffected.

To recapitulate—from the above statements, there seems to exist a relationship between the age of the legume selected, and the extent of modifications to be expected as a result of the action of the changed conditions. Those legumes subjected to new environmental conditions, while the tissues were yet plastic though quite undifferentiated, responded readily in all respects. The result was remarkable, both morphologically and histologically, for the legume as well as its contents. When, however, the legume was allowed to develop for a time under its ordinary surroundings, then the changes were found to vary in proportion to the state of development which had been attained before the new conditions were given an opportunity to act.

In this connection it may be well to point out that subterranean flowers or their young legumes, if kept growing in the air, will produce legumes with contents which are typically aerial, but such pods are always small, and contain only one seed. These statements are based upon experiments made with certain shoots which grew from the axils of the simple leaves (first pair of green leaves) of a plant. These shoots normally trail on the surface of the ground, and give rise to flowers which produce subterranean legumes. Certain of them were tied so that they were compelled to grow upward to the height of four or five feet. Upon the secondary branches which were thus forced to mature fruit above ground, were borne legumes of the character described.

These experiments are striking illustrations of the remarkable influence exerted by environmental conditions upon portions of *Amphicarpæa* possessing absolutely the same structure in the young state. When one is exposed to light, and the other to darkness, totally different morphological results are obtained. Doubtless there are other factors which are quite active in effecting such transformations; chief among these may be reckoned the amount of moisture. The great ease with which chlorophyll is replaced by anthocyanin, and the disappearance of the strengthening tissue, lead to the conclusion that the primitive hereditary characteristics may readily be set aside, and some latent or recently acquired ones be stimulated into vigorous development.

The great increase in size of the seeds is probably due to rapid accumulation of moisture. Weighing these seeds before and after exposure to heat, is proof that water forms a large percentage of the constituents. The more numerous my observations, the more I incline to the supposition that the hairs upon the legume must assist greatly in the work of imbibition, but as yet no definite information has been obtained.

I have stated in the published paper already referred to, that I consider the purple flowers to represent the original type. This remark is equally true of their legumes. Therefore taking these as a standard, we are ready from what we know concerning the structure of the subterranean flower, to anticipate equally great reductions in the subterranean legumes, and such we have seen to be the case. Developing in darkness, the protoplasm remains less active, the plastids decrease in number, the elements upon whose mechanical activity dehiscence in the aerial type is largely dependent, are entirely undifferentiated. Yet experimental work convinces one, that it would be an easy task to obtain a complete series illustrating every step in the transformation.

The history of *Amphicarpæa* is thus a striking epitome of flower and fruit variation. It is evident that the production of these diverse forms lies quite within the possibilities and indeed the probabilities of any one plant. These modifications occur before our eyes, as it were, in a comparatively short period of time.

To obtain evidence upon the subject of variation, it is usually necessary to compare many plants or perhaps to undertake a series of experiments, frequently repeated during many years ; even then the data may be neither satisfactory nor convincing to the investigator. A strong and plausible hypothesis may be advanced, but the difficulty is to obtain direct and sure proofs.

Considering the various types of flower found upon specimens of *Amphicarpæa* growing in the woods, or upon their borders, the opinion might be advanced that there are distinct species, some of which never produce purple flowers and their legumes. But the experimental evidence is conclusive proof, that one plant is capable of bearing all varieties of flower and fruit. Yet we cannot determine why in its native haunts, *Amphicarpæa* may produce all three forms of legume, sometimes but two, or again but one.

The formation of the subterranean legumes has become a definite part of the plant's inheritance, and for many reasons may well be regarded as an acquired characteristic rather than as an example of indefinite variation, since it is not usual for plants to bear fruit in this manner. What factors originally operated to cause the production of this type of seed, and to fix the result as a habit, it is impossible to say ; but the transmission is now undoubted. Often, indeed, no aerial legumes are formed, and the reproduction of the species is dependent solely upon these subterranean seeds.

While then the germ-plasm cells transmit the tendency to form legumes, whose morphological and histological features differ so greatly from those of the original type, yet when it is considered with what ease and rapidity either type of legume will respond to changed environmental conditions, the centres of variation seem to reside in the somatoplasm, and gradually affect the reproductive substance.

Two flowers in the same stage of development may be artificially forced to produce legumes quite different in character. Certain extrinsic factors stimulate, for example, the cells which are destined to produce indurated tissue; other factors suppress any such tendency. Yet it has been shown that some intrinsic properties firmly hold their own, provided the special legume is allowed to attain a sufficient degree of development in a definite natural environment, before being subjected to a new one.

We are thus able to trace gradual transitions in the structure of the flowers of the legumes and of the seeds, while we are likewise able to prove experimentally what external agents greatly modify the results in various cases. It is evident, therefore, that the subterranean seed habit must have originated in response to some extrinsic conditions, and our observation of the plant in its native haunts must convince us that the above-named characteristic has now become an inherited one.

In conclusion I will call attention to another line of investigation which *Amphicarpæa* has presented. During the latter part of August, 1897, Professor Macfarlane observed in the neighborhood of Strafford Station on the main Pennsylvania Railroad (about twenty miles from Philadelphia), a number of plants which bore *white* flowers. He mentioned the fact to me, and some time later I visited the spot. The plants were then in fruit. Those legumes which resulted from the evident flowers (in this instance—white) were almost invariably four-seeded. The plants bore many of these, and likewise others which, from position and shape, I knew to be the product of green aerial flowers; these were almost invariably three-seeded. The plants extended along the roadsides and in the woods for about a mile.

After noting the above facts, I examined again very carefully the number of seeds in the legumes of the purple-flowered *Amphicarpæa*, and found that as a rule those legumes produced by the colored flowers contained three seeds, but those from the cleistogamic only two. The underground legumes of the Strafford variety are small, and pale in color; many are nearly pure white. As the character of the soil was not particularly good, I did not attach much importance to this fact.

During the summer of 1898, I raised in the University greenhouses, plants from seeds of undoubted *A. monoica* (purple flower), from *A. Pitcheri* which had been sent me from Iowa, and also plants of the white-flowered type. These were grown in the same kind of soil, and under precisely the same conditions of temperature, moisture, etc. Unfortunately, the plants did not flower as abundantly as I should have liked, but the same results in regard to number of seeds in the various legumes were again noticed.

The subterranean legumes of *A. monoica* and *A. Pitcheri* were large, thick, deep purple-pink in color, and varied from a half to three-quarters of an inch or sometimes an inch in length. No difference could be distinguished in the appearance of these legumes.

Those produced by the Strafford variety were small, more spherical, none exceeded a half inch in length, and they were strikingly colorless. Here and there, a legume showed a pinkish hue ; but as a whole, the fruits presented a pure white appearance as the underground parts of the plants were exposed.

It may be that we have here a new variety. I have noticed the following differences—but doubtless there are others. The Strafford form exhibits a want of dark purple hue in the stem, as well as in the flowers and legumes ; the aerial seeds too are much smaller than those produced from the purple flowers. The plant is less hairy, and possesses fewer axillary runners than the ordinary type.

My intention is to plant the seeds, both subterranean and aerial, and ascertain whether these peculiarities persist. In the coming summer, the plants of the purple-flowered and the white forms will be carefully compared.

Water Storage and Conduction in Senecio præcox, D.C., from Mexico.

(WITH PLATES VII AND VIII.)

By John W. Harshberger, Ph. D.

[Read before the "Society for Plant Morphology and Physiology" at Ithaca, December 29, 1897. Abstract published in "Science" January 28, 1898.*]

WITHIN a few miles of the City of Mexico on the northern slopes of the Sierra del Ajusco is an extinct lava stream, hardened into solid rock. This coulee of lava, known locally as the Pedregal, extends from the summit of a hill called Chitle, one of the peaks of the southern mountain chain, down into the Valley of Mexico to the edge of a suburban town, Tlalpam. Of volcanic origin, it covers many hundreds of acres, and is extremely rough and uneven. It is difficult to collect plants in such a broken and uneven country, resembling a sea, congealed at the moment of its greatest turbulence. The lava is full of cracks, blisters, caverns and sinks, produced during the process of cooling. It is raised into cones, presents most curious sinuosities, and is here and there broken down into rugged, jagged protuberances, as sharp and cutting as a knife's edge.

The Pedregal is a wild flower preserve, and the vegetation is peculiar.† In many of the rougher portions the trees are practically absent and their place is taken by several plants, which alone are able to grow under such sterile conditions. One of these plants in particular, gives character to the larger vegetation, and is a conspicuous object on all of the drier ledges. The stem has an upright habit with tufted leaves at the top of a bright green color, and it can be seen for a considerable distance, as a prominent object on the lava bed.

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* Since the above dates an article entitled "La Flore des Regions Arides du Plateau du Mexico" has appeared in the "Revue General de Botanique," (February 15, 1898,) by S. G. Seurat. The author confirms the statements made as to the growth of this plant on the lava beds of Mexico.—J. W. H.

† Harshberger.—"Science," N. S., vi, p. 569, October 15, 1897, and vi, p. 908, December 17, 1897. "Bulletin, Torrey Botanical Club," xxiv, p. 178, April, 1897.

It resembles several of the endemic plants of the Canary Islands. In the zone of succulents, on these islands, grow plants which have adapted themselves to the long dry season.* A typical collection of these plants in the geographic arrangement of the Berlin Botanic Garden, so struck the writer as to their resemblance to *Senecio præcox*, that the following list was made of the Canary succulents found there. The most noteworthy examples are the following: *Euphorbia canariensis, E. mauritanica, Sempervivum strepsicladum, S. urbicum, S. arboreum, S. holochrysum, S. Youngianum, Senecio anteuphorbium* and *S. Kleinia.* The Canarian species of *Senecio* (which are found on geologic formations entirely volcanic, as evidenced by the traces of former seismic activity, the exceedingly mountainous, broken and jagged nature of the land)† are remarkably similar in habit and appearance to *Senecio præcox* of the Valley of Mexico. Similar conditions of environment physiologically affect plants of the same, and even of widely divergent, genera in an identical manner.

Many other plants are known, which give character to particular portions of the earth's surface. *Cereus giganteus* is found in Arizona; along the tidewater strands of the tropical seas the mangrove is predominant; in the Floridan swamps, the bald cypress grows; on the deserts of the Mexican plateau, tree yuccas, and cacti abound; in the Kalahari Desert of South Africa, *Welwitschia mirabilis* (*Tumboa Bainesii*) grows; in

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* GRISEBACH.—"Die Vegetation der Erde," ii, p. 482–88, 1884.

* DRUDE.—"Handbuch der Pflanzengeographie," p. 393–94, 1890.

† ALICE C. COOK.—"A Sketch of the Flora of the Canary Islands." "Bulletin, Torrey Botanical Club," xxv, p. 351, 1898.

New Holland, *Xanthorrhoea hastilis* is peculiar; likewise in the limited confines of the Pedregal *Senecio præcox* is a prominent and conspicuous element of the flora, and is therefore of geographic importance.

Senecio præcox—the tree groundsel—was first described by Cavanilles (1794, Icones et Descriptiones Plantarum, iii, 23 t. 244), as *Cineraria præcox*, and this mistake was concurred with by Willdenow (Sp. Pl., v, 3 p., 2078), Sprengel (Syst. Veget., v, iii, p. 546,) and De Candolle (Hort. Genev., t. 7). De Candolle in his Prodromus (vi, p. 431) transferred the plant from the genus *Cineraria* to *Senecio*, thus correcting the mistake into which the Spanish priest and botanist had fallen. The plant is a native of Mexico, and according to Hemsley (Biologia Centrali-Americana ii, 246), it has been collected at the following places and by the following botanists: North Mexico, region of San Luis Potosi, 6,000 to 8,000 feet (Parry and Palmer, 540); South Mexico, Valley of Mexico (Bourgeau, 178); around Toluca (Andrieux, 295); Guanajuato (Hertweg, 123); Tlalpam Pedregal (Harshberger); without known localities (Mairet, Moçino, Bates, Pringle).

Morphology.—The plant reaches, when fully matured, a height of three or four feet and usually stands upright withwithout any lateral branches (Plate VII, Figs. 1 and 2). These, however, occasionally appear at the side and by their growth soon overtop the main stem, or the stem may bifurcate in such a manner as to present a wide, open fork (Plate VIII, Figs. 1 and 3). The stem is cylindrical and from an inch in ordinary specimens to nearly two inches in diameter in the larger ones. It is fawn colored in the living state, the coloring being due to the smooth, corky bark which envelops it. The surface is marked closely by prominent crescent-shaped leaf scars, by large warty-looking lenticels, and by dormant buds, which may start into activity at any time, although they appear in most specimens dry and wrinkled (Plate VII, Fig. 3). The outline of a typical plant is broken in many cases by the branches aforementioned, which may arise from the sides, and after forming an elbow-shaped joint, grow up parallel with the main stem (Plate VIII, Fig. 3). In other cases, the branches have been broken off, and their rounded stumps are found covered with the weathered balsam which exudes. The smooth stem harbors a number of epiphytes, among which may be mentioned several species of *Tillandsia.* The growth of the epiphytes on the stem gives a somewhat venerable and hoary aspect to the plants covered by them. In their epiphytic growth, the *Tillandsias* simply embrace the host by their holdfast roots, and an inspection shows no corrosion of the cork by a ferment action, such as would happen if the epiphytic roots derived a supply of food from *Senecio præcox.*

The base or collar of our plant is usually swollen or rounded (Plate VII, Fig. 1). The roots come off from the lower surface of this rounded base, and are several in number.

The plant is surmounted by a dense crown of green leaves (Plate VII, Figs. 1 and 2), which appear after the commencement of the rainy season in Mexico (June to October). The leaves are developed from the apices of the branches only, are deciduous on the long terete petioles, cordate, much acuminate, sub-hastate, five to seven-lobed (Plate VII, Fig. 2). The lobes are very acuminate, spreading ; the lower ones deflexed. The texture is between membranaceous and fleshy.

Before the rainy season begins, when the leaves appear, in the months of March or April, or during the dry season, the corymbs of yellow composite flowers occupy the apex of the branch and bear several elongated, partially bracteolated, yellow branches, thickened below the capitulum.*

The structure of the stem is, however, what interests us most in this plant. It is succulent, easily cut across, and presents in the living specimens, a very watery, firm internal pith, a small cylinder of wood, a wide cortex with chlorophyll, and receptacles from which exude a resinous or balsamic substance. While botanizing in Mexico during the summer of 1896, plants in all stages of growth were gathered and pickled in a barrel containing one per cent formalin.*

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* CURTIS.—"Botanical Magazine," 3d ser., vol. X. t. 4803. 1854.

Looking at a cross section of a stem at least an inch and a quarter in diameter, which has been preserved in formalin, one is struck by a small disc-like area in the centre of the pith, a trifle over a quarter of an inch in diameter. In longitudinal section, the depressed circular area is seen to correspond to lens-shaped spaces, between the watery lamellæ or discs of pith, one-eighth of an inch or more in thickness (Plate VIII, Fig. 1). The pith cells are large, nearly twice as long as broad, and are filled with water (which is evidently cellular water), because in the formalin material, the protoplasm was found balled in the centre of the cell. The discs of pith are highly turgescent in the living plants (Plate VIII, Fig. 1), and a considerable amount of water is thus stored up, whether in combination with a mucilage or an organic acid could not be ascertained. When the pith is pressed the water exudes in small drops. The peculiar translucency of the cells indicates a large liquid content. The wood and medulla are very intimately connected together. The cortex can be removed from the stem with ease, leaving the wood and pith closely united. It is extremely hard to sever this connection without tearing the pith. As will become evident when the histology of the stem is presented, a number of the xylem bundles run a considerable distance into the pith, and thus bind the medulla to the woody cylinder.

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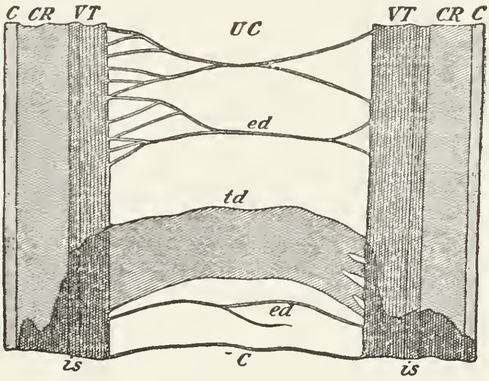
* Examination of the above mentioned material at this writing, December 24, 1897, sixteen months after collection, shows that all of the plants thus preserved are practically unchanged; a high encomium to pay a preservative liquid which stood in an open barrel covered by a lid and a cloth for that length of time. Botanists, therefore, who go to the tropics, should carry formalin, in preference to alcohol and the ordinary felt driers, because specimens preserved in formaldehyd can be dried and mounted for herbarium purposes with but slight loss of color upon return to civilization.

A question which very naturally arises in studying this plant is: How is the water prevented from being lost at the surface? The answer is found in the structure of the cortex. The bark consists of tabular cells arranged in a number of layers outside of the cortex proper, which is green to a considerable depth. Prominent in the cortex are reservoirs filled with a balsam-like fluid which hardens upon exposure to the air. These balsam receptacles are found in two positions in the cortex, under, near the phelloderm, in the exocortex, and next the bast in the endocortex. When a section is cut, the liquid exudes, hardens and thus closes the wound. Aeration of the stem is accomplished by the lenticels.

Upon a priori grounds, the pith stores up water in *Senecio præcox* during the rainy season for the use of the plant during the dry period, when the flowers shoot forth and the seed is produced at the expense of the reserve water. Have we evidence that such is the procedure?

Fortunately, a fully developed stem (Plate VII, Fig. 3) was collected on August 27, 1896, on the Pedregal near Tlalpam. It was carried to Philadelphia in a trunk and laid aside in the dry state for a number of months. Upon examination, it was found to have sprouted. Short lateral branches with undeveloped leaves soon appeared, and for the last sixteen months without water or soil, the growth has gone on, until at this writing, December 24, 1897, the dry stem shows at least four green branches (Plate VII, Fig. 3; Plate VIII, Fig. 2 a), turgescent and tipped by a number of small green leaves. These four branches measure respectively 1 ½, 1 ¼, 1, ⅜ inches.

Sections of this dry stem show the cortex region to be practically as turgescent as when first collected, the balsam exuding when a section is cut. The wood, too, appears normal in color and somewhat moist to the touch. The pith, however, is dry; the water has been removed from the discs, which are dry, paper-like and extremely brittle (Plate VIII, Figs. 2 a and 2 b). The spaces between the successive discs, or lamellar partitions of pith, have increased to such an extent as to form chambers one above another throughout the stem (Figs. 2 a and 2 b). Water is again absorbed, if the stem is immersed in that liquid, as I proved after the dry stem had been cut, so that the pith again swells up and assumes a nearly normal aspect. One remarkable exception, however, was found to the general dry appearance above described. At the very base of the stem, right at the cut off extremity, where one would least expect to find it, separated by two membrane-like diaphragms *ed* from the end *LC*, was a single, watery, turgescent disc *td*, as shown in the accompanying figure. The reason for the presence of the single turgid disc was not hard to find. The proper conduction of the water from the disc as from the other discs was prevented by the injury which the adjoining wood cells sustained, as indicated by their somewhat dry and brown appearance (Text Fig. *is*).



From a long series of careful and painstaking experiments beginning with those of Hales in 1727, we know that water and other crude substances travel up through the alburnum of stems. The elaborated materials on the other hand are carried down through the phloem, or bast portion of the stem. In *Senecio præcox* during the sixteen months that elapsed after the piece of stem was collected, the water was gradually baled out of the pith discs, or reservoirs, and was carried to the growing point (Plate VIII, Figs. 1 and 2). The leaves in ordinary green plants lose daily large quantities of water by transpiration, and the vessels which have to supply the leaves must make up the deficiency by receiving water from below.

An examination of Plate VIII, Fig. 1, will show the appearance of the pith discs as they occur in the turgescent condition. That it takes but a short time to refill the emptied discs was demonstrated by placing pieces of dry stem (Figs. 2 a and 2 b) in water. At the end of twenty-four hours, the dry membrane-like discs of the medulla imbibed water and swelled to about their original size as in Fig. 1. It would, therefore, appear that a single heavy rain would be sufficient to replenish the water, which it takes the plant months, or even a year, to consume.

Ludwig * in giving a résumé of our knowledge of xerophytes says: "Eine Anpassung an die trockensten Wohngebiete (mit oft ¾ Jahr lang anhaltender Trockenheit, stellen die Fettpflanzen oder Succulenten dar, bei denen durch besondere wasserspeichernde Gewebe das Wasser während der kurzen nassen Jahreszeit in grösserer Menge aufgesammelt und bis zur nächsten Nässeperiode angesammelt wird. Man hat diese Gewächse verglichen mit dem 'Schiff der Wüste,' dem Kameel." Professor Warming † gives us an instructive classification of water storing cells and tissues in a large number of plants, but in no place does he lay especial emphasis on the pith as a storage centre. Henslow‡ speaks of the pith without examples being given. He says: "In stems, the cortex and medulla act as storage tissue." Goebel,§ referring to succulent plants in general, makes this statement with reference to *Kleinia articulata*, a composite plant, where water is stored up in the pith: "Das Wasser ist theils im saftigen Rindengewebe, teils im Marke enthalten (letzeres z. B. sehr auffallend bei *Kleinia articulata*), und die Wassermengen, welche in dem Vegetationskörper einer solchen Pflanze aufgespeichert sind, sind teilweise recht beträchtliche."

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* Ludwig.—"Lehrbuch der Biologie der Pflanzen," p. 177, 1895.

† Warming.—Knoblauch, "Lehrbuch der œkologischen Pflanzengeographie," p. 199, 1896.

‡ Henslow.—"Origin of Plant Structures," p. 77, 1895.

§ Goebel.—Pflanzenbiologische Schilderungen," I, p. 54, Fig. 24, p. 55, 1889.

Histology of the Root.—The smallest roots ($\frac{1}{16}$-inch diameter) show about twelve concentric rows of cork with the outer wall of each cell somewhat bowed. The cortex consists of at least six concentric rows of rounded parenchyma cells, which when stained with Kleinenberg's alcoholic hæmatoxylon take a deep purple color. The central stele is diarchic, and differs little from that of an ordinary dicotyledonous root. The older roots show considerably more cork, and the outer layers have begun to exfoliate. Such roots soon exhibit the modifications, which take place in the increased thickening of dicotyledonous roots,namely the gradual shifting of the radially arranged phloem until it occupies an outside position with reference to the wood. When this growth is completed, normal increase proceeds as in the stem. Balsamic reservoirs are seen in two positions in the exocortex and endocortex of the roots.

Histology of the Stem.—The cork (*C* Text Fig.), in comparison to the diameter of the stem, is not so plentiful as in the roots. It is formed from a phellogenetic layer just outside of the green phelloderm. The green portion of the cortex shows quite large, light green chlorophyll grains. The spaces in the cortex, which are formed lysigenously, contain a fluid which, subjected to the following tests, seems to indicate that we have to deal with a balsam, rather than a true resin. Absolute alcohol dissolves the exudate without a sediment. Turpentine dissolves the clear, amber-like substance with a slight granular sediment. Ether dissolves it without a sediment. The exudation is insoluble in cold, but soluble in hot, potash, leaving a slight cloudy precipitate. Aqueous solution of acetate of copper was used upon several sections, exposed to its action for five or six days, without obtaining the emerald-green resin reaction. The smell, suggesting the presence of a volatile oil seems also to point to the exudate, being a balsam.

The phloem patches of the stem are more or less confluent, forming a cylindrical zone about the wood. The wood (*VT* Text Fig.), shows wide open elements, many of which extend as wings, or as wedges of growth, into the pith. Pitted and spiral elements are present, the latter internal. The bundles do not all extend so deeply into the pith ; only the primary or first-formed bundles do so, their protoxylem dipping into the pith cylinder.

A surface examination of the lower and the upper epidermis of the leaf reveals no peculiarities of importance. The absence of stomata on the upper, and their presence on the lower, surface is noticeable. They are quite large with crescent-shaped guard cells, each with a large nucleus. There are no trichomic structures on the leaf. A cross section reveals the absence of a true palisade tissue, the mesophyll being of rounded cells containing chlorophyll and clearly placed together without large intercellular spaces. The stomata open into narrow chambers, which are in communication by narrow connecting passage-ways. There is, therefore, but little of the so-called loose parenchyma. The stomata are not sunken, nor peculiar, although the guard cells are of a good size. These anatomical peculiarities show us, that as regards the roots and the stem, *Senecio præcox* is well protected against the dry season and can lay up a store of water in the pith for use during the period of drouth. The leaves, as they appear in the wet season, do not show the typical xerophytic structure, as one would naturally expect from their general leathery texture.

That *Senecio præcox* is well adapted to grow under the condition of climate presented in the Valley of Mexico needs no further proof than what has already been presented. One is always impressed in studying the vegetal kingdom by the different methods adopted by plants in securing the same end. The cacti of Mexico, and other succulents of that region, secure immunity from drouth by consolidation and by reduction of transpiration surface, as does likewise our plant, the tree-groundsel.

The Structure and Development of Internal Phloem in Gelsemium Sempervirens, Ait.

(*WITH PLATE IX.*)

By Caroline B. Thompson, B. S.

THE following is the result of observations made during the winter of 1897–98, in the Botanical Laboratories of the Biological Department of the University of Pennsylvania. The material used consisted of specimens of varying age, preserved in alcohol, which had been collected by Professor Macfarlane, while on a trip to Wilmington, N. C., and of seedlings grown in the greenhouses of the department from seeds collected by him. An abstract of the observations upon the stem was read at the meeting of the "Society for Plant Morphology and Physiology," held at Ithaca, N. Y., in December, 1897.

General Literature.

In the early years of the present century much confusion existed in regard to the terms for the softer elements of a vascular bundle. These were variously called bast fibres, bast cells, latticed cells, sieve fibres, etc. Hartig, in 1837, was the first to correctly describe such elements as sieve tubes, and to regard them as the essential constituents of the phloem. Several years later, Hartig's observations were confirmed by von Mohl, Nägeli and Hanstein. The investigation of plants with internal phloem, or phloem on the inner margin of the wood, was begun by Hartig in 1854, and continued by others. The orders Cucurbitaceæ, Asclepiadaceæ and Apocynaceæ were among the first to be studied. In 1875 de Bary originated the term "bicollateral bundle," a name that has been objected to by many of the later workers. From that time onward the number of investigators and the detail with which the work has been carried out have steadily increased. The most important contributions to the literature of this subject have been made by Vesque, Weiss, Russow, Petersen, Van Tieghem, Fischer, Scott, Gérard, Hérail, Lignier, Leonhard and Lamounette.

Various views are held by different writers upon the relation between the internal phloem and the other parts of the bundle. Some believe with de Bary that an actual bicollateral condition exists, and that the internal phloem is as much a part of the bundle as the external, and is of similar origin. Others, notably the French botanists Hérail and Lamounette, believe that the internal phloem is independent of the bundle and of different origin.

The following papers have been specially consulted:

Solereder, H. Ueber den systematischen Werth der Holzstructur bei den Dicotyledonen, 1885.

Scott and Brebner.—On the Anatomy and Histogeny of *Strychnos.* Annals of Bot., Vol. III, 1889.

Scott and Brebner.—On Internal Phloem in the Root and Stem of Dicotyledons. Annals of Bot., Vol. V, 1891.

D. H. Scott.—On Some Points in the Anatomy of *Ipomœa versicolor.* Annals of Bot., Vol. V, 1891.

Hérail.—Recherches sur l'Anatomie comparée de la Tige des Dicotylédones. Ann. des Sc. Nat., Bot. Sér. VII, T. II, 1885.

Lamounette.—Recherches sur l'origine morphologique du Liber Interne. Ann. des Sc. Nat. Bot., Sér. VII, T. XI, 1891.

LITERATURE RELATING TO GELSEMIUM.

Gelsemium sempervirens is commonly known in the Southern States as the "Yellow Jessamine," and is placed in the order Loganiaceæ by Solereder, Engler and Prantl, and Gray; in the order Apocynaceæ by Baillon, Le Maout and Decaisne.

In the Laboratory Contributions from the Biological Department of the University of Pennsylvania for 1884, J. G. Shoemaker has a few notes on the stem of *Gelsemium*. He remarks the widening of the medullary rays, and "the tendency of the pith to be penetrated by several plates of large thin-walled cells, which divide the pith more or less perfectly into four portions."

Professor Rothrock, in February, 1885, made a short verbal communication to the Philadelphia Academy of Natural Sciences concerning this stem. His attention was attracted by the fact that the diameter of the pith is greater in a very young twig than in a stem four times its size. He notes the presence of the four medullary phloem patches, and their encroachment upon the pith area.

A great deal of work has been done upon *Gelsemium* from a chemical and pharmaceutical standpoint, but its structure and development have not been thoroughly worked out. The root contains an alkaloid gelsemin, which is very poisonous, but is a valuable medicine when taken in proper quantities. The medicinal properties of *Gelsemium* were accidentally discovered about the middle of this century. An interesting account of the discovery and the primitive method of extracting the poisonous principle from the root is given by William Proctor, Jr., in the "American Journal of Pharmacy" for 1852.

Other records of the investigations upon the alkaloid gelsemin are to be found in later numbers of this journal, and in the "Proceedings of the American Pharmaceutical Association."

Histology of a One-year-old Stem.

A transverse section, about 1 mm. in diameter, of an internode at the close of the first year's growth shows the following structure (Plate IX, Fig. 1). Externally are three to four layers of cork, still covered in places by the prominently ridged cuticle ; next is the cortex, consisting of a zone of parenchyma four to five cells deep, rich in protoplasm and containing abundant chlorophyll and starch grains. A ring of large sclerotic cells, which appear in longitudinal section as clear refractive fibres of considerable length, lies on the outer margin of the vascular bundle portion of the stem. The bundle cylinder consists first of a zone of external phloem about six cells deep. Most of the cells are still embryonic, with large nuclei and abundant protoplasm, some few have differentiated into sieve tubes. In longitudinal section the sieve plates can be recognized. The septa are large, transversely placed, and bear either four or three sieve plates with numerous perforations. The cambium layer is clearly defined by its regular brick-shaped cells with large nuclei.

The wood is a broad zone, occupying more than a third of the area of the section, and is traversed radially by the oblong, deeply pitted cells of the medullary rays. A longitudinal section through the wood shows numerous spiral tracheæ in the inner or protoxylem region ; external to this are both short and long tracheids, whose walls are thickened and deeply pitted. Large vessels are numerous in the outer portion of the zone.

On the inner side of the wood lie four large rounded patches of internal phloem extending into the pith. These patches are two to three times broader than the external phloem zone, and consist also of sieve tubes and undifferentiated phloem elements. The inner margins of the phloem patches are bounded by a two-celled layer, which may be termed a phloem sheath (Plate IX, Fig. 1, *p. s.*). This is sharply differentiated alike from the adjoining pith cells and from the phloem. A row of somewhat similar but smaller cells separates the outer margin of the phloem patches from the wood, and immediately internal to this row are the patches of medullary cambium. The cambium cells have the usual brick-like shape, thin walls and large nuclei. The cells of the sheath are rounded and in close contact with each other. They have thickened pitted walls and are conspicuous by their size and the large amount of chlorophyll and starch they contain. The pith cells are much larger, have thin but slightly pitted walls, and a scanty supply of chlorophyll and starch, while the intercellular spaces are larger than those of the phloem sheath. A few short sclerenchymatous or "stone" cells are sometimes present. Very early in the life history of the stem death of the pith cells occurs. The cell contents dry up, the pith as a whole shrinks away from the sides and becomes detached from the phloem sheath, but persists as an inert somewhat lignified mass, until its place is usurped by the enlarging phloem patches.

HISTOLOGY OF THE STEM FROM THE SECOND TO THE TENTH YEAR.

In a transverse section of a stem at the end of the second year's growth, the most prominent change is the increased size of the internal phloem patches. Each has pushed farther out into the pith, and as the growth has been greater in the middle than at the sides, the inner margin has a curved outline, with the convexity toward the pith. The formation of new cells from the medullary cambium takes place centrifugally, the newly formed cells lying external to the old. On the inner side of each patch, adjoining the phloem sheath, a dark crescentic mass of partially obliterated tissue is now evident. This is composed of the older sieve tubes that have collapsed and been pushed together by the pressure from the new elements laid down by the active medullary cambium.

The external phloem has increased but little in breadth, in comparison with the internal patches, but the total number of cells and the actual area of the zone is greater than before. Here and there along the border are darker areas, composed of four or five compressed cells, showing that the same crowding and obliteration goes on, although to a less extent than in the internal patches.

In older stems, the increased size of the internal phloem patches becomes more and more prominent. The masses of crushed tissue, or "Hornbast," (Plate IX, Fig. 2) are more numerous and broader, the later formed ones lying in concentric layers external to the older masses. Some large phloem parenchyma cells are often present between the crushed masses, for they are better able to resist the crushing process, owing to their greater turgidity. The patches may thus present a stratified appearance from the alternation of the bands of crushed tissue and the scattered parenchyma cells. Each of the four patches usually divides into two parts, so that in the oldest stems eight cone-shaped masses of internal phloem are present. The neighboring patches grow together laterally, while they continue to encroach upon the pith. In the oldest stem examined (Fig. 2), of about twelve years' growth, the internal phloem patches entirely fill the former pith area, except a very small space in the centre, where a shrunken thread of dead tissue represents all that remains of the pith. The patches by this time are composed almost wholly of "Hornbast." Only a few sieve tubes are distinguishable, and these are more or less distorted. The contrast between the large cells of the phloem sheath and the dark crushed masses is very striking.

The breadth of the external phloem, which during the first few years was less than that of the internal patches, increases greatly in older stems. In a six year old stem its breadth almost equals that of the patches, in a ten year old stem it exceeds them. The same alternation of bands of "Hornbast" with parenchyma cells occurs as in the internal patches, but as the pressure conditions are different here the bands are narrower and less marked. As the growth has been centripetal, the newly formed tissue lies internal to the old.

The widening of the medullary rays is very noticeable in older stems. The width of a ray at the periphery of the wood is six or eight times greater than at the centre. Elongated cells, that are continuations of the rays, separate the cone-like masses of the external phloem zone.

HISTOLOGY OF A NODAL SECTION.

Near a node the circle of wood and external phloem becomes elliptical, and the patches of internal phloem lie at the ends and sides of the ellipse. The end patches are considerably larger than the side ones and are further divided into a central and lateral portion, the former for the petiole, the latter to remain in the stem. Higher up, the ends of the ellipse curve out more and more, and soon separate from the sides to form the petiolar bundles. Each bundle is accompanied by a portion of the internal phloem, so that at first the petiolar bundle is composed of external phloem, wood and two small masses of internal phloem. Left in the stem are the two long lateral curves of wood and external phloem as before. The two small groups of internal phloem that remained behind at each end now move together to reconstitute the end patches. Above the node the wood reunites into a continuous ring, while at the next node above, the leaf bundles will be given off from the opposite sides of the stem.

The petiolar bundles are at first distinctly bicollateral. Numerous patches of external phloem border upon the outer or lower face of the wood, and on its inner or upper face are two clearly defined patches of internal phloem. Almost immediately after the petiole has separated from the stem, the main petiolar bundle gives off two small lateral branches. These bundles consist chiefly of external phloem with a little xylem. They continue upward through the petiole and along the sides of the leaf, where their branches anastomose with branches from the main leaf bundle. A remarkable change soon takes place in the main petiolar bundle of a kind which, so far as I am aware, has not previously been described. Just above the point where the lateral petiolar bundles branched off, *the two internal phloem patches, one after the other, pass downward and outward through the wood to join the external phloem.* In a transverse section of a petiole, the phloem strands may be seen in longitudinal section passing between the xylem cells.

They bend outward along a radius of the bundle, and in a definite position, at about one half of the distance from the periphery to the mid-line of the bundle. After the passage of these strands, there is no further trace of internal phloem in the petiole or leaf.

HISTOLOGY OF THE ROOT.

The structure of a very young root, in transverse section, is illustrated in Plate IX, Fig. 3. The loose-celled starch-bearing cortex, about seven to eight cells deep, is separated by a thin-walled endodermis from the axial vascular cylinder. The bundle is typically diarch. The two groups of the protoxylem consist each of about six spiral tracheæ, and between them at the sides of the bundle lie two small patches of phloem, separated from the protoxylem by the procambium, a layer of large prominently nucleated cells. Outside the xylem and phloem elements, and just within the endodermis, is the pericambial zone. Later, by secondary growth, the xylem is united into a central cylinder, surrounded externally by a ring of phloem, but internal phloem is entirely absent in the root.

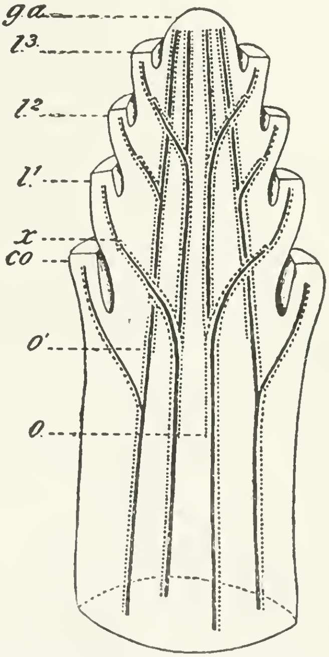
On older roots irregular warts or swellings are frequently found, which, when sectioned, reveal a vigorous fungoid growth. The fungoid hyphæ ramify through the cells of the inner and especially the middle cortex, and in some places large cavities occur, resulting from the breaking down of the cortex cells. These are filled with the coiled hyphæ and the fructifications of the fungus. Starch is usually absent in the cells inhabited by the fungus. In the root of a seedling about six weeks old, the fungus was already well established in many cortex cells.

HISTOLOGY OF THE SEEDLING.

The diarch condition of the root is continued in the hypocotyl, and it may at once be stated that the median plane of the two protoxylem masses corresponds to the median plane of the cotyledons. The spiral tracheæ of each end have at first a Y-shaped arrangement, the arms of the Y pointing toward each other, thus, —< >—, but as the hypocotyl increases in age, the cells of the arms move apart, taking a lateral position, with the phloem external to them, usually two patches to each side. This is illustrated in Plate IX, Fig. 4.

As differentiation proceeds more spiral tracheæ are interpolated between those already formed, so that a continuous ring of protoxylem is finally present. The phloem consists of small patches of finely divided cells, along the outer margin of the sides of the wood, but is not yet continued around the ends. At the level of the cotyledons, the phloem from the sides bends toward the ends, and the zone is thus completed. No recognizable internal phloem could be distinguished in the young hypocotyl.

In an older hypocotyl, in which secondary growth has gone on for some time, the fundaments of two internal phloem patches may be observed just below the cotyledonary node. The round hypocotyl becomes elliptical, preparatory to the separation of the cotyledons. Five or six large embryonic cells appear on the inner side of the wood. Their nuclei are large, and take a darker stain than the adjoining cells. In short, the fundament of an internal phloem patch has arisen in the leaf trace bundles, destined for the first, third, fifth and succeeding pairs of leaves. No such fundament is demonstrable in the pair of bundles for the cotyledons, second, fourth and succeeding pairs of leaves. With increased age these embryonic cells become a mass of small, finely divided cells, so that evidently the bundles for the odd pairs of leaves each possess an internal phloem patch. Throughout the lower and middle portions of the epicotyl, or the internode above the cotyledons, the opposite bundles are devoid of internal phloem, but just below the node bearing the first pair of leaves, two groups of embryonic cells appear in them, representing the fundaments of the internal phloem patches for the even pairs of leaves. When the node bearing the second pair of leaves is reached, all four patches of internal phloem are clearly distinguishable. In the bending out of the leaf bundles into the petioles, the same crossing of the internal phloem to the exterior takes place in the leaves of the seedling, that has been described above for adult leaves. Scott in his work upon *Ipomœa versicolor* found that in the hypocotyl near its junction with the root, the internal phloem passed through the xylem and joined the external phloem. He was thus able to prove the continuity of the phloem throughout the plant. Similar phenomena were observed by Gérard in different plants. There is no trace of any continuity between the external and internal phloem of the hypocotyl of *Gelsemium*. The course of the bundles throughout the hypocotyl and stem is indicated in the diagrammatic figure below.



o, origin of first pair of internal phloem patches; o^1, origin of second pair; *co*, cotyledon, l^1, first foliage leaf; l^2, second foliage leaf; l^3, third foliage leaf; *ga*, growing apex, *x*, crossing of internal phloem to exterior.

ORIGIN OF THE INTERNAL OR MEDULLARY PHLOEM.

In the growing apex of the stem the first cells to differentiate from the primary meristem are the spiral tracheæ of the protoxylem, which are arranged in radial rows. On their outer border appear groups of very small, thin walled cells, whose division walls lie in all planes. Soon thereafter similar groups of small cells are differentiated on the border of the pith area. These represent the internal phloem patches. The course of the internal phloem has been traced in older stems into the petioles, so it may be regarded as an integral part of the leaf trace bundle. It owes its origin to the same primary meristem that gives rise to the external phloem, and the protoxylem. Certain primary meristematic cells on the inner face of the protoxylem represent the medullary cambium. To the later activity of these cells the secondary growth of the medullary phloem is due. A radial arrangement of the later-formed medullary phloem cells is to be observed, and is an indication of their cambial origin. The medullary phloem appears in the hypocotyl some time after the differentiation of protoxylem and external phloem. Its origin, however, is from embryonic cells that are a part of the original primary meristem of the bundle. The appearance of these embryonic cells, on the inner side of two bundles in the hypocotyl, at a definite point below the cotyledonary node, and of similar cells in the two opposite bundles in the epicotyl, just below the first leaf node, may be explained as follows on phylogenetic grounds. Internal phloem is a secondary character acquired during the evolution of the plant. Since the hypocotyl and cotyledons are embryonic structures representing the primitive stages of growth of the plant, characters that have been acquired by, and are adapted to, the adult stem, may reasonably be found absent throughout the whole, or a part, of the hypocotyl. In this plant the lower portion of the hypocotyl exhibits the ancestral condition in the absence of internal phloem. The upper portion of the hypocotyl and of the epicotyl are transition stages, for two bundles have acquired internal phloem, while two bundles are as yet devoid of it. The region of the first leaf node shows the acquired condition of the presence of internal phloem in all four bundles.

The physiological significance of this acquisition, and the causes that led to it, are not clear. It is a noteworthy fact that internal phloem appears only in parts of this plant where pith is present. Although present in the stem, internal phloem is absent throughout the greater length of the petiole. It is present in the upper portion of the hypocotyl, but is absent in the lower part where the pith area is becoming constricted by inward growth of the xylem. Both internal phloem and pith are absent in the root. In plants like *Strychnos*, whose roots possess medullary phloem, pith is always present.

The view may be advanced, that to utilize the pith area, either for more perfect protection of the phloem, in these twisted and at times contorted stems, or to increase the total amount of it, a portion of the external phloem, during the evolution of the plant, dipped in from the bases of the petioles, through the fissures formed by the leaf traces in the vascular cylinder, and became internal in position. The climbing habit of this plant may be one of the factors in its evolution.

SUMMARY OF RESULTS.

1. The internal phloem arises primarily as four longitudinal strands, which are an integral part of the leaf trace bundles.
2. The origin of the internal phloem is simultaneous with, or slightly later than, the protoxylem and external phloem, so that the leaf trace bundles are bicollateral from the first.
3. The internal phloem patches are bounded internally by a two-celled phloem sheath.
4. The internal phloem patches grow centrifugally by means of a medullary cambium, the inner and older layers in time becoming crushed and obliterated.
5. Death of the pith occurs early in the first year.

6. The continued disintegration of the pith and growth of the internal phloem results in the filling up of the pith cavity with the latter.

7. The internal phloem, which runs into the petiole, constitutes there at first a bicollateral bundle system, but at the base of the petiole, it descends through the xylem as two strands, and from this point upward the primitive collateral bundle system prevails.

8. No internal phloem is present in the root.

9. A copious fungoid growth is found in the cortex of the root. Absorption of starch usually results in cells inhabited by the fungus.

10. No internal phloem is present in the lower portion of the hypocotyl, nor in the cotyledons.

11. Two of the internal phloem patches of the stem arise just below the cotyledonary node, the other two just below the node bearing the first pair of leaves.

12. Internal phloem is an acquired characteristic of the plant, and has probably been developed in these long and at times twisted stems, to supplement the external phloem.

EXPLANATION OF PLATE IX.

Fig. 1. Transverse section of one year old stem. × 35°.

Fig. 2. Transverse section of portion of ten year old stem, showing the internal phloem divided into eight patches. × 50°.

Fig. 3. Transverse section of young root. × 50°, cu, cuticle; ep, epidermis; co, cortex; end, endodermis; phl, phloem, prx, protoxylem.

Fig. 4. Transverse section of young hypocotyl. × 50°.

The Structure of the Cork Tissues in Roots of Some Rosaceous Genera.

(*WITH PLATE X.*)

By Martha Bunting, Ph. D.

IN his paper upon "Plant Hybrids"* Professor J. M. Macfarlane noted the presence of intercellular spaces in the cork region of the roots of *Geum urbanum* and *G. rivale.* While I was studying at the University of Pennsylvania during the summer of 1895, he suggested that I should try to ascertain whether the existence of intercellular spaces in the cork region was a definite character of the roots of *Rosaceous genera.* Since the summer of 1895 the study has been continued at intervals at the University of Pennsylvania and at the Woman's College of Baltimore. I wish to extend my thanks to Professor Macfarlane for his ever ready assistance and criticism of the work, as well as his very generous supply of material from the Botanical Garden of the University.

In addition to the paper already noted, I have found reference to intercellular spaces, in the cork region of *Rosaceous genera*, only in the following quotation taken from Vines' text-book of Botany, published in 1895: "The cells of the periderm are not always completely suberized. In some cases (roots and stems of *Onagraceæ*, *Hypericaceæ*, some *Rosaceæ*, etc.) some layers of the periderm consist of cells with a suberized zone like that of the cells of the endodermis, though these cells usually become completely suberized eventually. In other cases (*e. g.*, stem of *Poterium*, *Alchemilla*, *Agrimonia*, *Epilobium*) the periderm consists mainly of cells with cellulose walls, between which intercellular spaces are formed, together with occasional compact layers of cells with a suberized zone."

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* Trans. Roy. Soc., Edin., Vol. xxxvii, 1892.

The points presented in this paper will be given under the following headings:

(*a*) Intercellular spaces and annular arrangement of cell layers.

(*b*) Suberization of the periderm.

(*c*) Presence of nuclei in cells of the cork region.

(*d*) Starch in the cork region.

(*e*) Pigment of the cork region.

(*f*) Comparison of root and shoot.

(*g*) Evidence as to the relative evolutionary position of the *Rosaceous* groups.

(*h*) Summary of results.

(*a*) *Intercellular Spaces and Annular Arrangement of Cell Layers.*—The following groups of the *Rosaceæ* have been studied, with a view to solving the problem of the presence or absence of intercellular spaces, with the appended results:

Group Potentilleæ.—Ten species of this group have been studied, viz.: *P. norvegica*, *P. chrysantha*, *P. alpestris*, *P. geoides*, *Geum atrosanguineum*, *G. album*, *G. nutans*, *G. triflorum*, *Fragaria indica* and *Waldsteinia geoides.* All the above species of this group have been observed to contain intercellular spaces, which are quadrangular in shape. A noticeable character of the cork region of this group is the annular arrangement of the cell layers. These may be arranged according to three types: First, alternating layers of cells of different sizes as in *Geum album;* second, alternating uniseriate layers of cells whose cavities contain pigment, and of cells devoid of pigment contents, as in *Waldsteinia geoides;* third, uniseriate layers of flattened compact cells, with deeply pigmented cell walls whose cell cavities contain a brown pigment, alternating with less compact multiseriate layers which contain no pigment within the cell cavities, but which may sometimes have their walls pigmented, especially in the older layers at the outer margin of a section, as in different species of *Potentilla* and in *Fragaria indica.* This arrangement is shown for *Potentilla norvegica* in Plate X, Fig. 1. In *Fragaria indica, Potentilla chrysantha, P. geoides* and *P. alpestris,* the alternation of pigmented uniseriate and unpigmented multiseriate layers is not so marked a feature, especially if material is obtained during the late autumn, winter or early spring months. After treatment with iodine and sulphuric acid, however, the annular condition is very clearly defined. This may also be the case with some species of *Geum.* In *Geum atrosanguineum* (Plate X, Fig. 2), quadrangular intercellular spaces are present. The alternation as to the size of the cells in the uniseriate layers is not so clearly shown in this species as in *G. album, G. urbanum* and *G. rivale,* but proof that the cell walls in the alternating layer are suberized, is obtained by the use of sulphuric acid and iodine.

Group Agrimonieæ.—The following five species have been examined: *Agrimonia Eupatoria, A. parviflora, Alchemilla pyrenaica, A. pubescens, Poterium Sanguisorba* and *Acæna reptans.* Intercellular spaces are present in the cork region; and, as in some species of the last group, the cork is arranged in flattened compact uniseriate layers alternating with multiseriate layers of less compact cells. This condition is illustrated in *Agrimonia Eupatoria* (Plate X, Fig. 3); *Agrimonia parviflora* (Fig. 4), and in *Alchemilla pyrenaica* (Fig. 5). As regards intercellular spaces, the most remarkable arrangement is found in *Agrimonia parviflora.* The cork region of this species may be described as follows. Immediately external to the cork cambium is a single layer of compact flattened cells with brown pigmented cell walls, while a brown pigment fills the cell cavities; outside of this are three layers, the cells of which are so arranged as to form radiating beaded spokes. The inner two, of each triplet of cells in this zone, is of medium size, the external one is large, while between the radiating cell triplets are large intercellular spaces. The walls of each multiseriate zone may or may not be slightly pigmented, but there is no pigment within the cell cavities. The innermost compact layer and the adjoining zone of three layers seem to constitute a year's ring of cork; one, two or three additional rings may occur outside of the above described, according to the size and age of the root. These differ from the youngest only in the larger size of the most external cell layer and of the intercellular spaces. In a young root there may be from two to three zones in which the cells of the multiseriate layers are oval and of about equal size. As the root grows, the outermost cells of the multiseriate layers increase very much in area, with the result that intercellular spaces of peculiar shape and great size are formed. The cells of each uniseriate layer appear to increase in number by radial division, even after partial suberization has taken place. The evidence for this statement is, that very frequently a suberin lamella is found surrounding two cells, these cells being separated radially by only a cellulose wall with no indications of suberin; while a cellulose lining continuous with the radial wall is found upon the tangential walls of both cells. This apparent division of the cells in the uniseriate layer has also been observed in the following species: *Poterium Sanguisorba*, *Agrimonia Eupatoria*, *Geum album* and *G. nutans*. Division of the nucleus has not been seen, since the toughness of the tissues in the periderm region is so great that I have not been able to obtain satisfactory paraffin sections.

Group Spiræeæ.—The only species of this group studied was *Spiræa Filipendula*. This furnishes a beautiful example of the annular uniseriate layer with granular pigment contents, alternating with multiseriate layers. All the cell walls may be pigmented, while relatively large quadrangular intercellular spaces are present. The cells of the innermost zones are regular and of medium size, while those composing the outer sloughing region are much larger and irregular (Plate X, Fig. 6).

In the cork region of *Acæna reptans* very minute intercellular spaces are present; the elongated quadrangular cells are arranged in multiseriate layers of large and small cells, the former situated toward the exterior margin, the latter toward the centre of the section. The roots studied were young, and had only two multiseriate layers, each containing four rows of cells. The annular arrangement is also noticeable from the fact that the inner multiseriate layer is composed of cells whose walls are devoid of pigment, while the outer layer is composed of cells whose walls contain pigment.

Group Rubeæ.—In *Rubus occidentalis* and *R. villosus* very minute rectangular intercellular spaces are present, but they are not arranged with the regularity that is characteristic of the genera of the foregoing groups. Pigment is present in the contents of the outer sloughing-off layers, although in the sections which I studied the flattened uniseriate layers with pigment were not found. When stained with aniline green these layers appeared to be differentiated, the contents of a uniseriate layer being stained more deeply than those of the multiseriate layers.

Group Roseæ.—In the only species of rose examined, the cork was composed of flattened quadrangular cells; the whole region consisting of alternating layers of cells with and without pigment contents. The interior and exterior tangential walls of the non-pigmented layers are curved outwards, so that the pigmented layers have their cells compressed. The intercellular spaces are minute and are arranged irregularly as in the Group *Rubeæ.*

Group Pruneæ.—In *Prunus virginiana* the cork cells are flattened and tabular in shape. They are uninterruptedly connected with each other, so that there is no trace of intercellular spaces. The layers of cells are all uniform in appearance, and thus division into annular rings, which has formed so conspicuous a feature of the preceding types, is not observed.

Group Pomeæ.—The following four species were selected for study: *Eriobotrya japonica, Pyrus communis, P. japonica, P. Malus.* All of these genera show compactly arranged cork cells with no intercellular spaces. There is no separation into annular zones with the exception of the cork region of *Pyrus japonica.* In *Eriobotrya japonica* the cells have the usual tabular form found in this group, but the radial walls are much thickened, giving to them the characteristic appearance of endodermal cells; the cell walls also are deeply pigmented in specimens studied during early September. In *Pyrus Malus* the cell walls of specimens studied in early September are not pigmented, but within the cell cavities beautiful golden pigment is found.

The marked arrangement of the cork region into annular rings, each of which may again be made up of dissimilar rows, has led to the belief that these may each represent a year's growth. Since the vascular bundles do not so clearly indicate in roots as in stems the age of the root, this point has not been definitely settled. It is of interest to note that in the roots of *Geum atrosanguineum, Spiræa Filipendula* and *Agrimonia parviflora* studied in December, the uniseriate flattened layer is the one which is always found next to the phellogen, indicating that this layer is the last formed during the year.

Hartig and Sanio for the birch stem, and De Candolle for *Quercus Suber* have pointed out the fact that the number of zones of cork correspond with the number of years to which the stem has attained.

(*b*) *Suberization of the Periderm.*—With a view of ascertaining the amount of suberization of the different annular layers of the periderm or cork, many chemical tests have been used, among which may be mentioned iodine and sulphuric acid—in the proportions suggested by Russow—alcannin, chlorophyl extract, potassium hydrate, Schultze's solution, osmic acid, double staining with ammonia fuchsin and aniline blue, also chromic acid. In the groups *Potentilleæ, Agrimonieæ, Spi-*

ræceæ and *Rubeæ* the results of all the above tests indicate that the youngest uniseriate layer is composed of cells which early show suberized radial walls, and feebly, if at all, suberized tangential walls. In the older uniseriate layers, both radial and tangential walls are suberized, although there usually is a delicate cellulose lamella within the suberized wall. The youngest multiseriate layer is made up of cells whose walls are mainly formed of cellulose. These may or may not have a delicate lamella of suberin surrounding the cellulose; this suberin lamella usually increases in amount in the older layers of the section. In some cases, however, cellulose may be present in cell walls even after several annual rings have been formed. Thus cellulose has been noted in *Alchemilla pyrenaica* in the fifth multiseriate layer; while both in this species and in others the multiseriate layers may show suberization at a much earlier period. In the species of rose studied, the cell walls of the entire cork region are made up of suberin as well as cellulose lamellæ, while in the species studied of the groups *Pruneæ* and *Pomeæ* the cell walls are strongly suberized. If the cell walls are pigmented the color must be extracted, before characteristic cork tests with alcannin and chlorophyl extract are obtained.

In the application of all of these tests, an oak stem was used as a control, so that there might be reasonable certainty that the chemicals employed were such as would give the characteristic reaction with a substance known to be cork. All the tests employed gave excellent results with the oak stem, but with the very delicately suberized groups of *Rosaceæ* the most satisfactory reactions were obtained by iodine and sulphuric acid. The use of chlorophyl extract, alcannin, osmic acid, etc., was far from satisfactory in these groups, although in *Pruneæ* and *Pomeæ* they proved excellent tests for the differentiation of the cork. The question naturally presents itself as to whether these tests are less delicate than iodine and sulphuric acid or "Schultze," or whether there is a different condition in various cork cells which show special reactions to certain reagents. This fact has been suggested as a possibility by De Bary, but since in the case in point the species of *Pruneæ* and *Pomeæ* were uniform in their results with those from the oak stem, I am inclined to consider that they are not so useful as tests for cork when the amount of cork present is quite small.

(*c*) *Presence of Nuclei in Cells of the Cork Region.*—The stains which were employed for the purpose of demonstrating the nuclei were Bismarck brown, hæmatoxylin and picronigrosin. By the aid of these stains nuclei were found in *Potentilla norvegica*, both in the flattened uniseriate layer of cells and in the multiseriate layers, even so far as where the layers were sloughing off. In *Geum album* and *G. atrosanguineum* nuclei are observed in the outermost layer of large cells; when the periderm consists of five layers of cells, nuclei are also found in the layers of small cells in the first species. In *Fragaria indica* nuclei are present in the second multiseriate layer of cells. In *Waldsteinia geoides* nuclei have been noted in the eighth layer of cells of the periderm, the nuclei being found both in the cells with and without pigmented contents. In *Agrimonia Eupatoria* nuclei are found both in the uniseriate layer containing brown pigment, and in the multiseriate zones, and can be recognized even so far out as the eleventh row of cells. Nuclei are shown in the phototype of *Agrimonia*, but this root is a comparatively young one. In *Agrimonia parviflora* nuclei are demonstrated in the third uniseriate layer, as well as in the third multiseriate one. In *Alchemilla pyrenaica* nuclei are found in the eighth row of the periderm, and in *Spiræa Filipendula* in the tenth periderm layer. In *Rubus occidentalis* nuclei are noted both in the uniseriate and multiseriate layers, being present even in the eighth row of the periderm. In *Rosa* nuclei are detected in the eighth row of the periderm; these may persist both in the cells containing pigment and in those devoid of pigment. In *Prunus virginiana*, *Pyrus Malus, Pyrus communis* and *Eriobotrya* nuclei were not demonstrated. In these species the cork cells were very small and surrounded by heavy cell walls, hence it was difficult to prepare sections, and thus reach definite conclusions as to the presence of nuclei.

(*d*) *Starch in the Cork Region.*—In making tests for cork, by the use of iodine and sulphuric acid, it was observed that starch was present in the cork region, hence iodine was used with the different Rosaceous species in order to determine in what species starch was present in the roots. As a result of this test, starch was found in all the Rosaceous roots. Among the herbaceous and shrubby species it is observed in large quantities; though a relatively larger amount is found in the younger layers of cells toward the cork cambium, than in the uniseriate flattened cells with pigment contents, or in the cells which are peeling off, although it may be found in all of these. In *Prunus virginiana, Pyrus Malus, P. communis* and *Eriobotrya japonica* starch is also found in the cork cells, although in the roots of these species the quantity is not so great as in herbaceous roots. As would be expected, the amount of starch varies with the time of year in which the roots are studied. In late autumn, winter and early spring the periderm cells are loaded with starch, while the amount is appreciably decreased in the late spring and summer. This fact seems to indicate that the starch is used by the root, and that the cork cells as well as the other cells are reservoirs in which it can be stored. It would be very desirable to make tests for sugar, when the growth of the root has commenced, in order to determine definitely whether the starch found in this region is a reserve or a waste product.

(*e*) *Pigment of the Cork Region.*—Many of the genera of *Rosaceæ* contain pigment in some or all of the cells, as well as in the cell walls, it is however most frequently found in the uniseriate flattened layer, where such an annular layer is present. In sections of *Pyrus Malus* all the cells contained a beautiful golden pigment, while the cell walls were not pigmented. I have not studied sections of the root of this species during different times of the year. In some species a difference can be traced in the amount of pigment according to the season of the year in which the sections are studied. As examples of this fact I would quote the conditions observed in *Agrimonia parviflora* and *Spiræa Filipendula.* In the former species, sections taken from roots collected in September showed very little pigment in the cell walls or cavities of the uniseriate layers, sections from those collected in December contained no pigment, while sections from roots collected in July had pigment alike in the cell walls and in the cell cavities of this layer. In the latter species, sections taken from roots collected in December and April showed cells almost lacking pigment in the cell walls and cell cavities, while sections taken from roots collected in July showed pigment in the cell walls of all the cells, as well as pigment in the cell cavities of the uniseriate layers. While I have attempted to determine the chemical nature of this pigment, I have not as yet obtained sufficiently definite results to draw conclusions.

(*f*) *Comparison of Root and Shoot.*—Not very many observations have been made along this line, but I will note the following: Intercellular spaces have been observed in the cork region of the rhizome of *Geum album*, *Rosa*, *Alchemilla pyrenaica* and *Rubus villosus* stem. In stems of *Pyrus Malus* and *P. communis* there are no intercellular spaces. In general it may be said that in those species studied, the cork region of the stem was more strongly suberized than that of the root as was shown by the use of the cork tests.

(*g*) *Evidence as to the Relative Evolutionary Position of the Rosaceous Groups.*—In treating the *Rosaceæ* as an order Dr. Macfarlane informs me that he regards the only workable scheme of evolutionary relationship in this order to be one that follows closely the following lines: Starting with the *Potentilleæ* as the most primitive group, we pass by tolerably direct lines to the *Agrimonieæ;* from this group or from ancestors intermediate between it and *Potentilleæ* there may have diverged on the one side the ancestors of *Roseæ*, and on the other those of the *Spiræeæ*. A more direct line would lead to the *Rubeæ*, the three just named in their highest examples having all reached about the same degree of differentiation. Lines of specializing development would lead respectively from the *Rubeæ* to the *Pruneæ*, *Amygdaleæ* and *Pomeæ*. In my investigations upon the cork region of the above Rosaceous genera, the degree of specialized development reached in the cork cells of the different groups would seem to indicate in this respect such a relationship of the groups within the order.

(*h*) *Summary of Results.*—1. Large intercellular spaces are present in the cork region of the herbaceous genera, smaller spaces in the shrubby genera of the order *Rosaceæ*. They are absent in the arborescent genera studied.

2. A marked characteristic of the herbaceous and shrubby genera of *Rosaceæ* is the annular arrangement of the cells of the periderm region. In the arborescent specimens studied, the annular arrangement is not a feature of the periderm region. Results obtained from the study of this annular arrangement, suggest that each ring corresponds to a year's growth.

3. In herbaceous and shrubby species, a notable feature is the presence of a uniseriate layer of cells in which a lamella of suberin is present in the cell walls, this may or may not be present in the multiseriate layers. In very young roots the condition noted in Vines' text book is sometimes found, namely that the radial walls alone of the uniseriate layers contain lamellæ of suberin.

4. Nuclei have been noted, alike in the uniseriate layers in which the cell walls and contents are pigmented and in the multiseriate layers. These nuclei have been observed in some regions in the cells of the eighth layer.

5. Starch is present in cork cells of all the Rosaceous genera

studied, smaller quantities are found in the uniseriate than in the multiseriate layers, and in the arborescent than in the herbaceous or shrubby genera. The amount of starch varies with the time of year in which the roots are studied.

6. Pigment is found in all the Rosaceous genera investigated. This may be present either in the cell walls and contents of the uniseriate layers, the cells of the sloughing-off layers, or throughout the cell cavities of all the cells. In the species studied, the amount of pigment differed according to the time of year in which the roots were obtained.

7. Comparison of the few species of root and shoot observed, indicate a similar structure of the periderm in each; when the stem is of equal age with the root the suberization is greater.

8. Results of these investigations upon the periderm indicate a possible evolutionary relation of the groups of *Rosaceæ.*

EXPLANATION OF PLATE X.

Fig. 1. T. S. root of *Potentilla norvegica*, × 300°.
Fig. 2. T. S. root of *Geum atrosanguineum*, × 300°.
Fig. 3. T. S. root of *Agrimonia Eupatoria*, × 300°.
Fig. 4. T. S. root of *Agrimonia parviflora*, × 300°.
Fig. 5. T. S. root of *Alchemilla pyrenaica*, × 300°.
Fig. 6. T. S. root of *Spiræa Filipendula*, × 300°.

Comparative Studies on the Rate of Circumnutation of Some Flowering Plants.

By Elizabeth A. Simons.

DURING the spring term of session 1897-98 the Senior and Post-Graduate students of the Botanical Department began, under the direction of Professor Macfarlane, a series of experiments on the circumnutation of stems, for comparison of the results with those obtained by Darwin* and more recently in the Botanic Garden of the University by Dr. A. Schively.†

The writer was asked to continue and extend these results, for five plants specially recorded by Darwin. These are *Convolvulus Sepium*, *Phaseolus vulgaris*, *Lonicera brachypoda*, (*L. japonica*), *Wistaria chinensis*, and *Humulus Lupulus*. Marks were made at frequent time-intervals on a plate of glass placed directly above the circumnutating tip, and permanent graphic records have been prepared from them. The tables appended to this paper have been compiled from these.

The shoots were carefully tied to a support, and in most cases three internodes were left free. The records extended over a period of about six months, and thus included times when the sun gave considerable differences of light intensity and of temperature. In this paper no account is taken of light intensity, though data are being gathered which indicate that this is a factor in circumnutation, as is also the relative hygrometric condition of the atmosphere. It is somewhat unfortunate that few details are given by Darwin as to environmental conditions, but the writer regards the relatively higher temperature that prevailed during her studies as the main factor in producing the accelerated movements, as compared with those obtained by Darwin.

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* Power of Movement in Plants.

† Bot. Contrib. Univ. Penn. Vol. I.

Convolvulus Sepium.—Young actively growing shoots of this species were observed in the greenhouses of the Botanic Garden for four days and nights by members of the class in continuous relays. Note was made of the temperature of the house, and of the prevailing atmospheric conditions outside.

A dark sky and low temperature (15 C.–19.5 C.) gave discouraging figures for the first three days. The quickest time made during the period was 1 hour 45 minutes; the longest, 4 hours 15 minutes; average, 2 hours 40 minutes. The twenty-fifth of the month was a clear day and the temperature ranged from 15.5 C. to 33.5 C., resulting once in a circumnutation in 57 minutes: the longest took 3 hours; the average time for the day was 1 hour 53¼ minutes. The behavior of the plants with respect to light and temperature would point to Darwin's conclusion, that both are important factors in plant movement.

The rainy days afforded no good opportunity for observation of periodicity of growth, but the clear day caused an acceleration of movement from 8.05 a. m. till 2.30 p. m. in one case, and from 9 a. m. till 4.32 p. m. in another, the successive circles being:

SPECIMEN 1.

First circle	2 hours	30 minutes.
Second circle	1 hour	45 "
Third circle	1 "	25 "

SPECIMEN 2.

First circle	2 hours.	
Second circle	1 hour	50 minutes.
Third circle	1 "	15 "
Fourth circle	1 "	10 "
Fifth circle	1 "	2 "
Sixth circle		57 "

The night circumnutations were longer than the average daily ones.

Later in the season, on July 18, August 8 and 15, experiments were performed on shoots of *Convolvulus Sepium* growing in the garden. The plant spread itself, for the most part, along the ground, slender branches, now and then, standing upright and exhibiting a tendency to climb. The times of revolution were as shown in the table appended, the shortest, 1 hour 11 minutes, being longer than the spring time of 57 minutes. This plant produces two distinct types of stem, the twining and the prostrate. The latter are of a darker color, more woody, thicker and have stronger tips. Two of these were tied to a support and watched from 8.30 till 1. There was no perceptible movement, later a slight one. Nothing further took place before 2.35 p. m., when observation ceased. Three days later no evidence of ability to climb could be noticed, the stems having been left tied to their supports.

Humulus Lupulus.—Winter buds of the Hop were transplanted and formed vigorous shoots by February 9, when circumnutation movements were observed during two days (two distinct plants used), with the appended results.

The periodicity of growth was not so marked in this case, but gave indication of afternoon acceleration, as will be seen by reference to the table.

Darwin's shortest time for the Hop was 2 hours 8 minutes, during what he called "hot weather." Our plants were first observed in late winter when 1 hour 5 minutes was the shortest period. On a hot day (July 15) tips of a plant growing in the garden were protected from wind, supported, and watched under glass; the time 1 hour 40 minutes, was obtained. Another tip made a circle in 2 hours 35 minutes, when it seemed to be burnt. On August 16, another tip was tried, with the result 2 hours 14 minutes. Casually observed, the tips do not seem inclined to grow vigorously in the hot weather. The quick spring movements confirm this.

Lonicera brachypoda and *Phaseolus vulgaris* were used in class work, but insufficient data were collected from which to generalize. Those obtained have been incorporated with the writer's later observations. The Scarlet Runner studied was a seedling, the *Lonicera* had grown from a cut-back plant.

A fine specimen of *Lonicera brachypoda* growing in the garden, and having no chance to climb, bore numerous healthy tips. It was surrounded by a box, whose top and bottom had been removed, as well as some boards from the side. This was partly covered by glass, but plenty of air was allowed entrance. Some of the shoots were so vigorous that they needed no support; others were long stems and had to be supported.

On July 18, no glass was used, but record was kept by marks on rods of wood laid above the tip. The movements then obtained were the slowest recorded, and it is likely that the extra protection from wind and retention of heat by the glass cover, produced the quicker movements of August 15.

When watched in the greenhouse, *Lonicera* showed itself extremely sensitive to heat; high temperature invariably being associated with rapid movement.

Our shortest time is 1 hour 43 minutes; Darwin obtained 7 hours 30 minutes. He makes no mention of temperature beyond saying "a warm room in the house." The full results obtained are appended.

Scarlet Runner seedlings were used in the greenhouse for experiment. The panes of the house were whitewashed, and the intensity of light thus diminished. The tips never needed support, young ones being invariably chosen. The results as appended show that the shortest time obtained with this species was 1 hour. On April 5, we obtained 1 hour 20 minutes, as compared with Darwin's observation on May 2 —1 hour 55 minutes.

Wisteria chinensis.—On July 11, young sprouts of this species were found in good condition on a cut-back plant in the greenhouse; one that had been entirely shorn of branches and leaves. Darwin worked on this species in a greenhouse from May 13 till May 25, and obtained as his shortest time, 2 hours 5 minutes, with an average of 2 hours 50½ minutes. By taking advantage of every fresh sprout as it began to reach for a support, the results appended were obtained.

Growing in a protected corner of the garden was another *Wisteria*, with long, strong stems, of which three were unsupported. On August 18, these were gently secured to stakes, two of them hanging just as on the vine, being secured at one point to a stick to insure against wind interference, the third being tied upright with about nine inches free. This began its twisting in that position, but gradually became horizontal from the point of attachment. Apparatus for marking was set up and the following results obtained. All the stems were exposed to direct sunlight except for a short time, when they passed through an arc shaded by the plant itself.

The shortest time obtained was 2 hours, the average of all observations 2 hours 15⅜ minutes.

As to the time of day when circumnutation is most rapid, the following diagram was made from the foregoing records of the five species examined. The temperature recorded for a circumnutation represents the average for the hours between which the circle was made. The temperature records are suggestive, those during the shortest circumnutations ranging from 25.5 C. to 36.5 C.; of the longest, 15.5 C. to 30.5 C.

From the table it will be noticed that of the 31 quick twinings, 22 are in the afternoon. Looking at the list of long circumnutations 8 of 18 are in the afternoon. But a more detailed analysis brings out some interesting results, that correspond very closely with those given by Dr. Schively in the paper already cited (pp. 296–97). The following statement is there made regarding periodicity of circumnutation. "Beginning with the early hours of morning, there is a gradual acceleration until 11 or 11.30 a. m. The greatest rapidity occurs from this time until 2 or even 3 p. m. After that time there is a gradual decrease in the rate, until several hours after midnight. The maximum period may be much extended, beginning earlier and continuing until 4 or perhaps 4.30 p. m., if the day is very hot." From the tables compiled by the writer, the following comparison of four-hour periods is obtained. The agreement of these statistics drawn from five species of plant, with the statement above quoted need merely be noted.

Between the hours	were performed
8—12 a. m	11 circumnutations.
9— 1 p. m	12 "
10— 2 "	11 "
11— 3 "	16 "
12— 4 "	19 "
1— 5 "	23 "
2— 6 "	18 "

When the experiments were begun, it was thought that under our comparatively bright sky and warmer temperatures, as contrasted with the atmospheric surroundings of England, circumnutations might be performed more rapidly than in the shortest time-limits given by Darwin. Subjoined is a comparison of the two sets of results:

AT DARWIN'S HOME.

Phaseolus.	(Greenhouse, May 2)	1 h. 55 m.
Humulus.	("Hot weather")	2 h. 8 m.
Convolvulus.		1 h. 42 m.
Lonicera.	(House, April 3)	7 h. 30 m.
Wisteria.	May 16	2 h. 5 m.

AT UNIVERSITY OF PENNSYLVANIA.

Phaseolus.	(Greenhouse,	April 5)	1 h. 20 m.
"	"	July 18	1 h.
Humulus.	"	February 9	1 h. 5 m.
Convolvulus.	"	March 25	57 m.
Lonicera.		April 5	2 h. 48 m.
"		August 15	1 h. 43 m.
Wisteria.		July 11	2 h.

CONVOLVULUS SEPIUM

1898. Date.	A M	A. M.	P M	P M	Hrs	Min	Temp	Cent		
Mar 22		9.00	1 15		4	15			Greenhouse.	Rain
			1.15	4.00	2	45			"	"
		11 15	1.30		1	45			"	"
			1.30	3.45	2	15	3.00 4.00	19.4 17.7	"	"
		11.00	1 10		2	10			"	"
			1 10	3.10	2	00			"	"
			3.10	5 10	2	00			"	"
		Night	7 10	9.45	2	35			"	"
			7.10	9.45	2	35			"	"
			7.10	9.20	2	10			"	"
Mar. 23	8.00	10.35			2	35	7 30	17.7	Greenhouse.	Rain.
		10.35	1.10		2	35	9.00	17.7	"	"
			1.10	4.00	2	50	11.00	17.2	"	"
	7.30	9.45			2	15	11.30	16.6	"	"
		9.45	1.30		3	45	12.00	17.7	"	"
			1.30	3.20	1	50	3.00	19.4	"	"
			3.20	5.20	2	00			"	"
	7.30	10.25			2	55			"	"
	8.00	10.20			2	20			"	"
		10 20	12.50		2	30			"	"

CONVOLVULUS SEPIUM.

1898. Date.	A. M.	A. M.	P. M.	P. M.	Hrs	Min.	Temp.	Cent.		
Mar. 23			12.50	3.00	2	10			Greenhouse.	Rain.
			3.00	6.00	3	00			"	"
Mar. 24	9.45		12.45		3	00	2.30	16.1	"	"
			12.45	3.45	3	00	3.30	15.5	"	"
			1.00	4.35	3	35	4.30	15.5	"	"
		Night	7.25	10.45	3	20			"	"
			7.20	10.45	3	25			"	"
	8.15	11.45			3	30			"	"
Mar. 25	8.05	10.35			2	30			"	Clear.
		10.35	12.20		1	45			"	"
			1.05	2.30	1	25			"	"
	9.00	11.00			2	00			"	"
		11.00	12.30		1	50	12.45	31.1	Sunny.	
			12.30	1.45	1	15	1.00	33.3	Cloudy	
			1.45	2.55	1	10	2.00	26.1	"	
			3.30	4.32	1	2	3.00	27.2	"	
			3.30	4.27	0	57	5.00	27.2	Clear.	
		Night	7.00	10.00	3	00		17.7		
			7.00	10.00	3	00		15.5		
			7.00	9.45	2	45				

CONVOLVULUS SEPIUM.

Date	A. M.	A. M.	P. M.	P. M.	Hrs	Min.	Temp.	Cent		
July 18			12.47	2.20	1	33	12.00 1.00	28.8 30.0	In garden.	
			2.16	4.25	2	9	2.00 3.00	30.5 31.0	"	
Aug. 8	10 00	11 11			1	11	10.00 11.00 12.00	29.5 30.0 31.8	In garden.	P. cloudy.
	9 15	11 11			1	56	1.00 2.00 3.00	32.2 33.0 32.2	"	"
Aug 15			1 15	2.30	1	15	1.00 2.00 3.00	26.6 28.2 28.8	"	"

LONICERA BRACHYPODA.

Date.	A. M.	A. M.	P. M.	P. M.	Hrs.	Min.	Temp.	Cent.		
Apr. 5			2.15	5.00	2	45	2.40 3.30 4.30	26.6 26.1 24.4	Greenhouse.	
	9.00	11.55			2	55			"	
July 18			12.00	3.00	3	00	12.00 1.00	28.8 30.0	In garden.	
			2.00	4.50	2	50	2.00	30.5	"	
			12.00	4.50	4	50	3.00	31.0	"	
Aug. 8	8.45	10.30			1	45	8.00 9.00 10.00	26.6 27.6 29.5 30.0	"	Short shoot
Aug. 15		10.20	12.55		2	35	10.00	24.4	"	Clear.
		11.15	1.00		1	45	11.00	24.4	"	--
		11.15	1.30		2	15	12.00	26.1	"	Short shoot.
		11.15	1 36		2	21	1.00	26.6	"	Long shoot.
			2.02	4.08	2	6	2.00	28.2	"	Hazy.
			2.02	3.45	1	43	3.00	28.8	"	"

HUMULUS LUPULUS.

1898. Date.	A. M.	A. M.	P M.	P M.	Hrs.	Min.	Temp.		
Feb 9	9.30	11.03			1	33		Greenhouse	Clear.
		11.03	12.20		1	17		"	"
		12.20	1.25		1	5		"	"
		1.25	2.46		1	21		"	"
Feb. 10	9.10	10.33			1	23		"	"
	10.33	11 53			1	20		"	"
		11.53	1.31		1	38		"	"
			1.31	2.48	1	17		"	"
			2.49	4.05	1	16		"	"
Feb. 9	9.30	11.32			2	2		"	"
		11.32	1.02		1	30		"	"
			1.02	2.25	1	23		"	"
			2.25	4.00	1	35		"	"
Feb. 10	9.08	10.30			1	22		"	"
	10.30	11.53			1	23		"	"
		11.53	1.35		1	42		"	"
			1.35	2.55	1	20		"	"
			2.55	4.20	1	25		"	"
July 15		11.20	1.00		1	40	Mean temp. for day: 28.8 C.	Outside.	
	9.10	11.45			2	35		"	
Aug. 16			2.06	4.20	2	14		"	

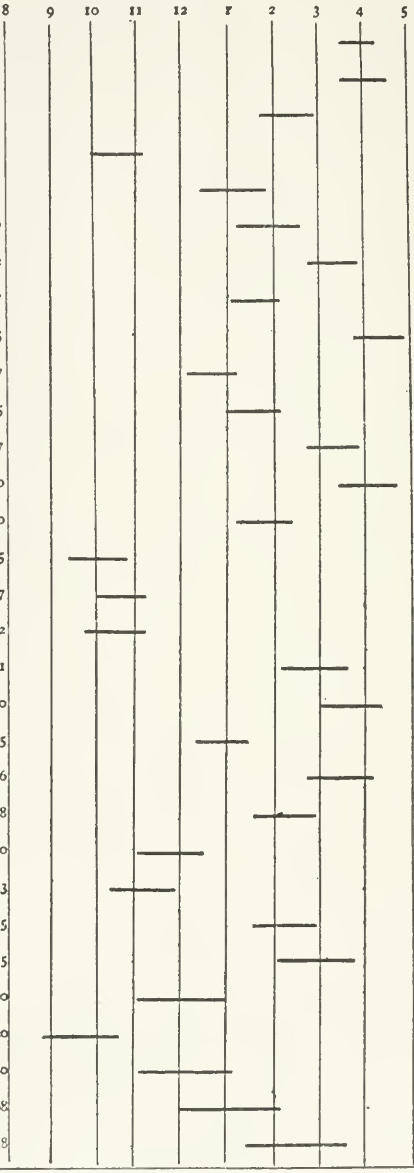
PHASEOLUS VULGARIS.

Date	A. M	A. M.	P. M.	P. M.	Hrs.	Min.	Temp	Cent.		
Apr 5	9 30		2 10		4	40	9.30 10.00 11.00	17.7 16.8 23.8		
			1 00	5.00	4	00	11 50 12.00 1.00	11.6 12.7 16.8	Greenhouse..	Very draughty.
			2 10	3 37	1	27	2.00 2.40	16 6 26.6		
			3 00	4 20	1	20	3.30 4.30	26.1 24.4		
Apr 4			3.00	4.45	1	45			Greenhouse.	
July 11	9.07	11 30			2	23		26.6	"	Clear.
July 15	9 55	11.12			1	17			"	P. cloudy.
	9 35	10.45			1	10			"	"
July 18			12.13	1 17	1	4		33.3	"	"
			1 00	2.05	1	5		33.3	"	"
			1 05	2 07	1	2		33.3	"	"
			2 14	3 31	1	17		33.3	"	"
			3.42	4.45	1	3		33.3	"	"
			2.42	3.47	1	5		33.3	"	"
			2.42	3.42	1	00		33.3	"	"
			3.31	4.40	1	9		33.3	"	"
Aug 8	10 00	11 17			1	17		36.6	"	"
			1.12	2 20	1	8		36.6	"	"

WISTERIA CHINENSIS.

Date.	A M.	A M	P M.	P M.	Hrs.	Min.	Temp. Cent.		
July 11	9.07	11 20			2	13	26.6	Greenhouse.	Clear
		11 10	1 10		2	00	26.6	"	"
July 18			2 14	4.45	2	31	33·3	"	"
Aug 15		11 20	1 35		2	15		In garden.	"
		11 20	2.05		2	45		"	"
			12.00	2.08	2	8		"	"
			1.30	3.38	2	8		"	Hazy

COMPARATIVE TABLE.



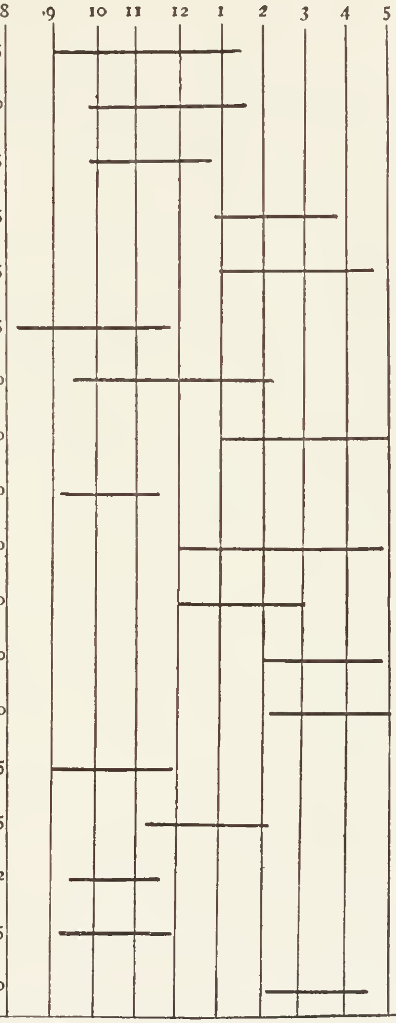
			Hrs.	Min.
Convolvulus -	3.30	4.27	0	57
"	3.30	4.32	1	2
"	1.45	2.55	1	10
"	10.00	11.11	1	11
"	12.30	1.45	1	15
"	1 15	2.30	1	15
Phaseolus	2.42	3.42	1	00
"	1.05	2.07	1	2
"	3.42	4.45	1	3
"	12.13	1.17	1	4
"	1.00	2.05	1	5
"	2.42	3.47	1	5
"	3.31	4.40	1	9
"	1.12	2.20	1	8
"	9.35	10.45	1	10
"	10.00	11.17	1	17
"	9.55	11.12	1	17
"	2.14	3.31	1	17
"	3.00	4.20	1	20
Humulus	12.20	1.25	1	5
"	2.49	4.06	1	16
"	1.31	2.48	1	17
"	11.03	12.20	1	17
"	10.33	11.53	1	20
"	1 35	2.55	1	20
Lonicera	2.02	3.45	1	43
"	11 15	1.00	1	45
"	8.45	10.30	1	45
Wisteria	11.10	1.10	2	00
"	12.00	2.08		8
"	1.30	3.38	2	8

Shortest circumnutations.

Forenoon, 9. Afternoon, 22.

COMPARATIVE TABLE.

			Hrs.	Min.
Convolvulus	9.00	1 15	4	15
"	9.45	1 30	3	45
"	9.45	12.45	3	00
"	12.45	3.45	3	00
"	1.00	4 35	3	35
"	8 15	11.45	3	30
Phaseolus	9.30	2.10	4	40
"	1.00	5.00	4	00
"	9.07	11 30	2	23
Lonicera	12.00	4 50	4	50
"	12.00	3.00	3	00
"	2 00	4 50	2	50
"	2 15	5 00	2	45
"	9.00	11 55	2	55
Wisteria	11 20	2.05	2	45
Humulus	9.30	11 32	2	2
"	9 10	11 45	2	35
"	2 06	4 20	2	14



Longest circumnutations observed

Forenoon, 10 Afternoon, 8.

Observations on the Development of Some Embryo-sacs.

(*WITH PLATE XI.*)

By R. E. B. McKenney, B. S.

THE study of the development of the embryo-sac was suggested to me in the fall of 1897 by Professor J. M. Macfarlane, and has been continued, with interruptions, until the present time. I wish to here acknowledge my indebtedness to him for suggestions and criticisms of my work. Plants of *Scilla hyacinthoides var cærulea*, *S. campanulata*, *Lilium tigrinum* and *L. candidum* furnished the material for study.

Methods.

As fixatives, I have employed Kleinenberg's Picro-sulphuric, Picro-acetic, Absolute alcohol, and Flemming's strong Chromosmo-acetic. The Picric solutions gave very good results. Objects not larger than a pea, and with unindurated tissue, will be fixed in an hour. The Picric solutions are best washed out with 50 per cent alcohol. As a clearing fluid I found cedar oil rather better than xylol, since it is less volatile, and seems less likely to cause shrinkage. Care must be taken, however, to have *all* the cedar oil replaced by pure paraffin, or good sections will not be obtained. The sections were cut on the Minot microtome with a feed of from 6 to 10 μ and fixed to the slide with Mayer's albumen. As staining agents I have used Delafield's Hæmatoxylin, Eosin, Guignard's Methylgreen-Fuchsin, Heidenhain's Iron Hæmatoxylin and Flemming's triple stain. A combination of Delafield's Hæmatoxylin and Eosin proved especially good.

EMBRYOLOGY.

The following descriptions refer principally to the two species of *Scilla* which differ but little from each other. The ovules of *Scilla* are anatropous and are arranged in two tiers, in each of the three cells of the superior ovary.

The ovules make their first appearance as small knob-like outgrowths from the placenta. No differentiation is discernible among the cells of these outgrowths. Soon one of the hypodermal cells grows larger than its neighbors, and is further distinguished from them by the rather denser consistency of its protoplasm and the larger size of its nucleus. This cell is the archesporium. Unlike that of the surrounding cells, the cytoplasm of the archesporium is usually free from sap vacuoles of any appreciable size. During this stage in the life of the archesporial cell, two to three large nucleoli are contained in its nucleus. By the time the archesporium becomes three to four times the size of its neighbor cells, the cells near the free end of the young ovule become raised in an annular welt-like fashion. This is the beginning of one of the integumentary coats, the *primine* (Plate XI, Fig. 1 p). The young ovule meanwhile continues to grow and bends slightly on its axis away from its fellow. The archesporium keeps pace in its growth with the ovule, its growth, however, being greater in the long than in the short diameter. The nucleus of the archesporium now divides, and by the deposition of a periclinal wall the archesporial cell is divided into two cells (Plate XI, Fig. 1). The smaller and outer of these two cells is the primary tapetal cell (Fig. 1 T). The formation of the primary tapetal cell is soon followed by another division of the archesporium into two unequal cells (Fig. 2). The smaller and outer cell remains passive and at a later period disintegrates. The lower and larger remains in an active condition.

About the time of the second division of the archesporium, the cells just within the primine become raised up to form a second annular elevation (Fig. 2 s). This is the *secundine* fundament. Following the formation of the secundine fundament the primary tapetal cell usually divides by a periclinal wall into two cells. By these successive divisions, the archesporium becomes pushed down into the tissue of the nucellus, so that, instead of lying just beneath the epidermis, it is separated from it by a chain of three cells (Plate XI, Fig. 3).

The archesporium now passes through a growth period of considerable duration. After the growth phase, the archesporial nucleus divides and the archesporium becomes divided by a periclinal wall into two equal-sized daughter cells. (Fig. 4). I have only been able to observe this division in the anaphase and telophase stages. The anaphase stages observed, however, were quite clear and the number of chromosomes could be readily counted. The number of chromosomes, which in this division enters into the formation of the daughter nuclei, is eight (Fig. 4 a). All the divisions of the surrounding nucellar cells and the first division of the archesporium show sixteen chromosomes. Hence it seems quite probable that this last division of the archesporium is the reduction division.

Of the five cells formed by the division of the primitive archesporium, the three outer remain inactive while the two inner grow actively until they become two to three times as large as the outer three. Attendant on this great growth of these daughter cells of the archesporium, is a gradual degeneration of some of the neighboring nucellar cells. The outer of these two daughter archesporial cells is the young embryo-sac cell. The nucleus of each of these cells divides, but no cell wall is laid down between the daughter nuclei. Thus two bi-nucleate cells are produced (Fig. 5). The inner bi-nucleate cell becomes passive for a time. The outer bi-nucleate (embryo-sac) cell, however, elongates until it becomes about twice its former length. The bi-nucleate cells again become active and by the division of each nucleus two quadri-nucleate cells are produced, the nuclei of the outer bi-nucleate cell dividing first (Plate XI, Fig. 6). The inner quadri-nucleate cell now becomes passive and after a time undergoes a slow degeneration, which becomes complete about the time of fertilization.

After a short resting phase the quadri-nucleate embryo-sac grows rapidly, and by the degeneration of the three cells first formed from the archesporium, as well as the surrounding nucellar cells, it comes to lie against the epidermis of the nucellus. By division of each of its nuclei, the quadri-nucleate embryo-sac at length shows eight nuclei. The eight nuclei of the embryo-sac now arrange themselves in the typical manner (Fig. 7). By the time the embryo-sac has become fully developed the ovule has become completely anatropous, and the primine and secundine have grown so as to completely cover in the nucellus.

The question now arises as to the nature of the quadri-nucleate cell next the embryo-sac. For some time it appeared to the writer that this cell might represent a sporocyte which had divided into four spores. Accordingly if such were the case, one would be led to suppose that the embryo-sac represented two sporocytes which were not separated by a cell wall. This view seemed to find some support in the observation by Mann of a partition wall in the embryo-sac of *Myosurus*, and in the possibility of the fertilization of the synergids as described by Dodel and Overton in *Iris* and *Lilium*. A more careful study of its relationship, however, seems to militate against the hypothesis that the embryo-sac contains two sporocytes. In its development, the Angiospermic embryo-sac agrees, on the whole, pretty closely with that of Gymnosperms. Further, the reduction division, which so far has been found to take place during spore formation, appears to occur in the formation of the embryo-sac and its neighbor quadri-nucleate cell. Accordingly it seems probable that the quadri-nucleate cell is an embryo-sac or macrospore which only goes part way in its development.

In the development of its ovule, *Scilla* may be said to differ from most plants in having a second macrospore undergo partial germination. In those species which develop more than one embryo-sac, each embryo-sac is developed as a rule from a separate archesporium. In *Scilla* the rudimentary second embryo-sac is developed from the same archesporium as the normal embryo-sac. Commonly it is the lowest cell of the chain of archesporial daughter cells which becomes the embryo-sac. In *Scilla*, however, the lowest cell of the chain forms the second rudimentary embryo-sac, while the normal embryo-sac is developed from the cell above this.

CYTOLOGY.

The larger part of my cytologic study was made on *Lilium* as its cells are much larger than those of *Scilla*. *Scilla*, however, shows essentially the same cell structure as *Lilium*. The cytoplasm of the archesporial cells of *Lilium* and *Scilla* reveals a well-marked reticulum or net-like structure, that of *Scilla* having the reticular threads more closely woven together. The reticular threads appear to be made up of very minute stainable granules embedded in a less stainable substance. At the point of crossing of the reticular threads there is usually seen a larger granule. In *Lilium* there can usually be seen a number of small bodies scattered irregularly through the cytoplasm which stain very much like the nucleoli. The cytolymph is comparatively small in amount in archesporia, but gradually increases during development, until it occupies the larger part of the embryo-sac.

The nucleus is always surrounded by a definite membrane which does not show any pores such as described by Mann. In some cases the chromatin seems to have a loose reticular structure, in others it appears in scattered irregular masses, and in still other cases it has the appearance of a thick twisted thread. The chromatin of resting nuclei has a homogeneous appearance, but during the early stages of mitosis it can be seen that the chromosomes are composed of a number of quite evident granules. This granular structure of the chromatin cannot be seen in the later stages of mitosis.

The nucleoli vary in number from one to as many as three in *Scilla* or seven in *Lilium*. The nucleoli are quite frequently surrounded by a clear zone filled with nuclear sap. The nucleolus has a dense homogeneous appearance and in *Lilium* may exhibit a number of small yellowish oil-like bodies embedded in its substance. In preparations stained with a single stain like Delafield's Hæmatoxylin the nucleolus stains much more deeply than the chromatin. If sections stained in Hæmatoxylin be now stained in Eosin the nucleoli become red while the chromatin still remains blue. This would indicate that the nucleoli are of a different chemical nature from the chromatin, since they readily part with one stain for another. The chromatin holds stains tenaciously. Hence if the nucleolus was simply a very condensed mass of the chromatin, we would expect that chromatin would the sooner part with its Hæmatoxylin for the Eosin. Such, however, is the reverse of what happens.

In some studies of mitosis in the Basidiomycetes, Wager describes the nucleolus as becoming smaller in size and the chromosomes increasing in size in proportion as the nucleolus becomes smaller. The nucleolus takes on a fainter stain as it becomes smaller; while the chromosomes, which originally stained differently from the nucleolus, now take on the same staining properties as the nucleolus originally possessed. He accordingly comes to the conclusion that the nucleolus is probably similar in nature to the chromatin. I have failed to observe anything which would tend to confirm this view.

The centrosome question, as far as the Spermatophyta go, seems almost as far from solution as ever. Since Guignard first described centrosomes in *Lilium Martagon*, quite a number of workers have applied themselves to this problem. One of the most recent papers is that by Mottier on *Lilium* and *Podophyllum*.

He has traced almost the complete history of spindle formation. He finds the spindle fibres to first make their appearance as a band of kinoplasmic fibres surrounding the nucleus. These later assume such a position that they radiate from the nucleus in all directions. The fibres then push into the nucleus, become attached to the developing chromosomes, and arranged in the form of a multipolar spindle. Then the fibres rearrange themselves to form the bipolar spindle. The spindle fibres seem but seldom to come to a definite point at the poles; they form usually a brush-like termination. At no period during the history of the spindle was Mottier able to find anything resembling a centrosome.

The writer has observed most of the stages as described by Mottier during spindle formation. At no period in the cell history is a centrosome visible. It seems quite likely that if centrosomes are present in flowering plants, they would be seen by a greater number of observers, especially when the same objects and methods are employed.

EXPLANATION OF PLATE XI.

Fig. 1. Longitudinal section of apex of young ovule of *Scilla hyacinthoides var. cærulea.* p, primine fundament; T, primary tapetal cell; A, Archesporium.

Fig. 2. Longitudinal section of an older ovule of *Scilla hyacinthoides var. cærulea.* p, primine fundament; s, secundine fundament; T, primary tapetal cell; A, Archesporium; a, first daughter archesporial cell.

Fig. 3. Longitudinal section of young ovule of *S. hyacinthoides var. cærulea.* T^1 and T^2, daughter cells of primary tapetal cell.

Fig. 4. Chain of cells resulting from division of primitive archesporium of *S. hyacinthoides var. cærulea.* A^1 and A^2, daughter cells resulting from last division of the archesporium.

Fig. 4*a*. Last division or reduction division of the archesporium giving rise to cells A^1 and A^2 of Fig. 4.

Fig. 5. Longitudinal section of ovule of *S. hyacinthoides var. cærulea* showing the binucleate daughter cells of the archesporium. A^1 becomes the normal embryo-sac.

Fig. 6. Chain of cells developed from primitive archesporium, *S. campanulata.* E, Embryo-sac in quadrinucleate stage.

Fig. 7. Mature embryo-sac with rudimentary second embryo-sac E^1 below, in *S. campanulata.*

Observations on some Hybrids between Drosera filiformis and D. intermedia.

By J. Muirhead Macfarlane, D. Sc.

(*WITH PLATE XII.*)

ACCOMPANIED by a few of my students, an excursion was made, during the third week of June, to the rich botanizing grounds near Atco, N. J. Amongst the pine-barren swamps of that locality was an area several acres in extent, that was partially flooded, but clothed with a profuse surface vegetation of swamp or bog plants. These consisted almost entirely of the four species, *Eriocaulon septangulare*, *Drosera intermedia*, *D. filiformis*, and a yellow-flowered *Utricularia*.

The later blooms of *D. filiformis* were still abundant, but the involute flower stalks of *D. intermedia* were just unrolling, and as was proved later, these did not bloom fully till the second week of July. Casting one's eye across the swampy mass of vegetation, the clusters of pale pink elongated leaves of *D. filiformis* contrasted strongly with the short, dense clusters of crimson-pink leaves belonging to *D. intermedia*.

After a considerable stretch of the marsh had been examined, my attention was arrested by a rather distant group of plants, somewhat intermediate in height and color between the two common species around. A nearer examination of the eleven plants composing the group, suggested the possibility of their being natural hybrids between the above-named species. They were carefully removed, without injury, to one of the greenhouses in the University Botanic Garden, where they have since been grown and watched. A continued and careful exploration of the swamp failed to reveal the presence of additional plants or plant clusters like those already found.

Detailed comparison of the leaves, flower stalks, inflorescence, flowers and period of blooming, still further confirmed the suspicion entertained on finding them. Histological investigation of the three, as well as of *D. rotundifolia*, which was only sparingly present in the marsh, shows that the last-named species does not contribute to the formation of the plants in question. It equally demonstrates a minute blending, in all parts of the hybrids, of the histological peculiarities of *D. filiformis* and *D. intermedia*.

When the eleven specimens were collected, care was taken to remove sods of both parent species, and all three were grown in neighboring flats in the greenhouse. The parent species matured an abundance of what seemed to be good seeds. The contents of the hybrid pods were apparently useless. A detailed description of the macro- and micro-morphology of each will now be given under the following heads: (*a*) leaves, (*b*) axis of inflorescence, (*c*) inflorescence, (*d*) period of blooming, (*e*) size and color of the blooms, (*f*) floral structure.

(*a*) *Leaves.*—The leaves of *D. filiformis* (Plate XII, Fig. 1) are on the average 8 inches long and $\frac{1}{12}$ inch wide. The statements made in current botanical manuals that there is "no distinction between blade and stalk," * also that they are "glandular-pubescent throughout," † are equally incorrect. A non-glandular portion ⅜ to ⅝ of an inch long is the petiole, and in the winter bud-leaves is the only part developed for protective purposes. The base of this non-glandular part is a flattened quadrangular area ⅛ inch by ¼ inch. It is densely tomentose-pubescent along its lateral and upper margin, as well as externally. The individual hairs vary greatly in size, in the number of cells composing each, and in the amount of their branching. While some consist of a few cells joined lengthwise into a long thread which may give off one or two attenuated branches, the majority are long flat ribbons that give off numerous narrow branches.

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* Gray's Manual of Botany, 6th edit., 1889.

† Britton and Brown's Illustrated Flora, Vol. II, p. 162.

The upper epidermal cells of this area are of varying shape and size, but average 150 × 38 μ. They contain relatively few scattered small chloroplasts, each 2.5–3 μ across. Stomata are not present over this area. Its outer or lower epidermis consists of longer but narrower cells, 185 × 20 μ, which are well filled with large chloroplasts each 7.5–8 μ across. Amongst these are a few stomata, each 40 × 23–25 μ. More abundant than the stomata are two-celled sessile glands of stoma-like character, each cell being filled with rich finely granular substance, and a highly refractive nucleus.

The leaves of *D. intermedia* (Plate XII, Fig. 3) are on the average 1 ½ inches long, and in the blade ⅕ inch wide. There is a sharp differentiation between petiole and blade, the latter becoming both widened out and glandular at its junction with the former. At the base of the petiole there is, as in *D. filiformis*, a somewhat quadrangular area, but here the edges slope toward the upper part of the area. It is glabrous throughout, except across the upper transverse boundary, where are 7 to 10 strong multicellular unbranched, or slightly branched, hairs. The epidermal cells are 225 μ × 28 μ and contain a very few small chloroplasts.

The lower epidermis of this area consists of elongated narrow cells measuring 200–225 μ × 15 μ. It has no stomata, and instead of the two-celled glands of *D. filiformis*, are glandular bifid hairs (Plate XII, Fig. 4*c*).

In the hybrid the leaves vary considerably according to age and position in the annual rosette, but comparison of its leaves with those of *D. filiformis* and *D. intermedia* prove that this variation is an inherited one from both parents. The earlier leaves of the annual rosette in *D. filiformis* are comparatively short and taper to a somewhat blunt point, the typical summer leaves may be 10–11 inches long and greatly attenuate at their tips. In *D. intermedia* the spring leaves decidedly approach in outline to those of *D. rotundifolia*, those of summer develop as the typical spathulate growths. We have here a key to the degree of variability seen in the hybrid. The first growths of a rosette consist of leaves which are 1¾–2 inches long, the glabrous petiole being ½–⅝ inch long, the remainder being lamina. The summer leaves are on the average 3½ inches long, of which ⅞–1 inch may be petiole. The basal quadrangular area is very averagely intermediate alike in size and shape between that of each parent. Numerous hairs, which are structurally identical with those of *D. filiformis*, occupy a like position, but they are considerably shorter and show fewer branchings. Across the upper transverse boundary a few rather stronger hairs stand out amongst the more flattened ones, as an inheritance of the few prominent hairs of *D. intermedia.* The upper epidermal cells are 188–200 $\mu \times 32\ \mu$, while the chloroplasts are very small and scarce.

The cells of the lower (outer) epidermis are on the average 186–195 $\mu \times 21\ \mu$, and the chloroplasts measure 2.5 μ. Numerous two-celled sessile glands are inherited from *D. filiformis*, but the bifid hairs characteristic of the other parent are also present, though considerably reduced in size.

It is not the intention to deal in this paper with the details of internal leaf anatomy, and accordingly a short comparison may now be made of the three forms, in their petiolar region, between the base and lamina. In all, the two-celled glandular or bifid hairs are abundant, and study of the Figures 4*a*, 4*b*, and 4*c* on Plate XII will graphically illustrate their relative size. In *D. filiformis* they are only slightly elevated above the epidermis on subjacent cells, and they measure 28 $\mu \times 18\ \mu$. In *D. hybrida* they are saccular elevations above the epidermis, and measure 33 μ high by 32 μ across. In *D. intermedia* the hairs are bifid bladder-like appendages each 45–50 μ high by 37 μ across.

Stomata are now present over the under surface of the three and show noteworthy differences. Those of *D. filiformis* are 38 μ long by 24 μ wide, and one guard cell is usually larger than, or somewhat obliquely placed to, the other, while a more or less evident sinus exists at their opposed ends (Plate XII, Fig. 6*a*). The chloroplasts of the guard cells are 20–22 in number, and measure 3.2 μ across. In the hybrid the stomata are on the average 32 μ × 25 μ, and show a very slight tendency toward inequality of size or position. (Fig. 6*b*). The chloroplasts are 15–17 in number and measure 2.5 μ across. In *D. intermedia* the stomata measure 26 μ × 25 μ; the guard cells are very neatly crescentic and fitted at their ends (Fig. 6*c*). The chloroplasts are 12–14 in number and measure 1.8 μ across.

The blade of the leaf is tentacular throughout on its upper surface. The general color of the lamina of the three forms is markedly different. Leaves of *D. filiformis* might be described as pinkish-green, those of *D. intermedia* as deep crimson green, while those of the hybrid are averagely intermediate to the naked eye. Microscopically examined, these differences are found to be due to the relative distribution of a rich crimson-red pigment, which is only present in the tentacular hairs. In *D. filiformis* it is wholly confined to the oval or elliptic head of each hair, there being a sharp contrast in color between the green top of the hair-stalk and the red base of the head. In *D. intermedia* the pigment is richest in the head, but is distributed in the cells of each hair throughout two-thirds of its length. In the hybrid the pigment is less rich than in the last, and extends throughout one-third to rarely one-half the length of the stalk.

The tentacular hairs of all three vary greatly in length, so that no exact comparison is possible. The club-shaped head of each in *D. filiformis* is broadly elliptic and is on the average 220 × 165 μ. The head of each hair in *D. intermedia* is ovate-truncate and measures 230 × 105 μ. That of the hybrid is elliptic-ovate in outline and measures 210 × 125 μ.

The upper laminar epidermis of *D. filiformis* consists of cells which are highly variable in size and shape. Stomata are abundant, as many as nine being in a circular area 300 μ across. Each stoma measures 40 × 30 μ. Four-celled sessile glandular hairs are also disposed over the epidermis. The stomata and glandular hairs of the lower epidermis are about equally as abundant as those of the upper epidermis. The upper epidermis of *D. intermedia* has on the average seven stomata over the above-named area, and each measures 27 × 22 μ. Two-celled glandular hairs take the place of the four-celled ones of the other parent. In the hybrid an average of eight stomata is observed in the above area, and each measures 34 × 25 μ. There is a curious admixture of the glandular hairs. Some are two-celled only as in the latter parent, others are four-celled as in the former, while not a few are three-celled owing to a median or somewhat oblique wall crossing one of the twin cells. On the lower epidermis the same peculiarity occurs, though in leaves examined the majority were two-celled as if the glandular tissue swayed toward one parent. Suggestive cytological considerations come up here of which we shall speak later. Chloroplasts occur in all epidermal cells, but these vary considerably in different cells and individuals, so that exact comparison seems impossible.

(*b*) *Axis of Inflorescence.*—Comparison of a considerable number of axes of the parents with the eleven of the hybrid indicates that the average height of that in *D. filiformis* is 9¾ inches, in *D. intermedia* 5½ inches, and in the hybrid 6¾ inches. Here it should be noted that the average heights given for the parents have been taken from plants growing in several localities, and that the hybrids do not seem to have attained the age vigor which may yet be expected. This may to some extent affect the length of the axis.

Surface views of the epidermis of the axis show a quite glabrous surface in *D. filiformis* with stomata in considerable numbers, each measuring 40–44 μ in length. That of *D. intermedia* is provided with stomata which are 35–36 μ in length, but about as abundant as these are the two-celled glands typical of the species. In the hybrid the stomata average about 38 μ, while the two-celled glands of the latter parent are reproduced in size, but reduced in number.

A slight reference may be made now to the structure of the cortex. In *D. filiformis* this is made up of 4–5 rows of thin-walled, richly chlorophylloid cells, with considerable intercellular spaces between. An indurated sclerenchyma zone next succeeds, composed of 5–6 layers of cells, which gradually become larger toward the interior. The amount of thickening is greatest in the second and third layers of sclerenchyma. The average thickness of the thickest walls is 7–8 μ. The chlorophylloid cortex of *D. intermedia* consists of 1–2 layers of cells with fairly large intercellular spaces between. Subjacent to this are 3–4 layers of slightly indurated, but mostly very large, cells from the second layer inwards. The average thickness of the walls is 3.5 μ. The outer cortex of the hybrid is made up of 2–3 layers of chlorophylloid cells, with relatively large intercellular spaces. Subjacent are four to five sclerenchyma layers, considerably less thickened than in *D. filiformis*, but which have, as in it, the most highly thickened zone in the middle of the mass. The average thickness of the thickest wall is 4.5 μ.

The fascicular system will not now be treated of, as equally in parents and hybrid it is complicated in arrangement.

(*c*) *Inflorescence.*—The scorpioid cyme of *D. filiformis* is, from the bud state onward, semi-erect, and the flowers are closely crowded along the false axis, against which they are closely adpressed. The number of flowers in an inflorescence averages 14. The cyme of *D. intermedia* is closely coiled in the young state, the component blooms are somewhat loosely arranged, they spread out in radiate fashion from the false axis and the average number of flowers is 8. The cyme of

D. hybrida is rather strongly incoiled, as in the latter parent. The blooms average 10, and these are set in a rather scattered sub-secund manner along the false axis.

(*d*) *Period of Blooming.*—In my paper on "Plant Hybrids"* I advanced a considerable body of facts to prove that the flowering period of many hybrids is very exactly intermediate between the periods of the parents, while other hybrids show a decided divergence toward one parent. It has not been possible to do more in the present study than to ascertain approximately the relative period of blooming. From a study made at several localities in New Jersey during the past season, it was learned that the first blooms of *D. filiformis* opened on June 7–10, and by June 28 the terminal flowers of the cyme were open. When collected on June 30, a number of the lower flowers on the more vigorous hybrid plants had already passed, while the lowest flowers on small plants and the lower middle flowers on strong plants were fully open. The plants continued to flower in the University greenhouses till August 3. As already noted, when plants of *D. intermedia* were gathered the involute flower stalks were just unrolling, and later observation showed that this species begins to bloom about July 3, and continues until about August 15. In attempting to account for the origin of the patch of hybrid plants, therefore, it seems extremely likely that a late bloom of *D. filiformis*, and an early bloom of *D. intermedia* had been concerned in the pollination process. The limited observations just recorded point to *D. hybrida* as a form which blooms at a period between those for the parent species. It should be said, however, that the writer and another member of the party succeeded in obtaining some four blooms of *D. filiformis* on as many plants as late as August 10. That they were entirely out of season was proved by hundreds of surrounding plants having their capsules in an advanced state of maturity.

(*e*) *Size and Color of the Blooms.*—Those of *D. filiformis* are on the average ⅞ inch across, though they vary more than do those of the other parent. They are of a purple-pink hue. The petals of *D. intermedia* are ¼ inch across and of a pure white color. Those of the hybrid seem decidedly to approach the latter, since the flowers are ⅜ inch across, and are white with a faintly recognizable pink flush.

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* Trans. Roy. Soc. Edin.. Vol. XXXVIII.

(*f*) *Floral Structure.*—The sepals form an exceptionally interesting study. Those of *D. filiformis* are abundantly covered externally with glandular hairs (Plate XII, Fig. 7*a*) which vary greatly in structure and length. They may be from 180–380 μ long. Generally it may be said that all of them end in a flat-topped mass of 4–12 glandular cells, but the stalk supporting these may be a single cell row or two rows below gradually running into one or two rows above. Each stalk also consists of 3–6 or 7 tiers of elongated cells. In the distal hairs of each sepal spiral tracheæ may enter the base of the hair and soon end blindly, or may be prolonged about one-third the length of the hair. Rosette-shaped, four-celled, sessile glands, and toward the base of the sepals two-celled glands of similar appearance, are also disposed over the exterior. Stomata are abundant, are nearly circular in outline and measure 30 × 28 μ. The internal (upper) epidermis is glabrous, and consists, like the upper epidermis, of very variable cells, alike in size and shape. In *D. intermedia* the outer surface of each sepal is glabrous, but a moderate number of two-celled glands on a slight stalk cell, as well as stomata, occur over its surface. In *D. hybrida*, the outer surface of each sepal bears the same type of stalked gland hair as is seen in *D. filiformis* (Fig. 7*b*), but here they are on the average only one-third to one-fourth the length of those on the parent species. But, like the parent, they vary in structure amongst themselves. Beside these are the four-celled sessile glands, which differ in no way as to size from the parent, each being 30 μ across. They are more scarce, however, than in the parent. We thus have the interesting case shown of one type of parental hair inherited in greatly reduced numbers and of much smaller size, and another type inherited in reduced numbers, but of exactly the same size. Still further, the hybrid inherits bilobed glands from *D. intermedia* in moderate quantity. Three types of epidermal glandular appendage are thus inherited by the hybrid from its parents.

I do not propose to describe in detail at present all the points of floral or fruit and seed structure. The careful observations of another season will be needed before exact comparisons can be made. It may be said, however, that the pollen grains of *D. filiformis* are richly granular, largest in size and measure 56 μ across. Those of *D. intermedia* are also granular and plump; they measure 44 μ across. Most of the hybrid grains are more or less starved or poor in protoplasmic substance. They measure 48–50 μ, so that development of the pollen grain walls has proceeded perfectly, though the enclosed fertilizing substance appears to be imperfect. The ovules and seeds of both parents matured well, those of the hybrid remained small and in most instances developed as empty or nearly empty shells. Cultivation and future study will demonstrate how far this may be a constant character.

The naked eye and histological details described above emphasize the position first fully established by the writer,* that a hybrid is, as a rule, down to its minutest details, a blended reproduction of both parents in which the morphological and physiological details of each are reduced by half. In no case has it been possible to detect the entire loss in the hybrid of some parental condition, and this cannot be too strongly insisted on in view of the loose theoretical reasonings often indulged in now on questions of heredity. Peculiarities of structure, and equally, it seems, of function, are not readily lost, but may persist, in a gradually reduced state, in several succeeding generations.

A glance at the comparative results, however, equally demonstrates that in this, as in some other hybrids studied, certain parts or organs tend more toward one parent than another. The balance of development throughout in the present case is evidently toward *D. intermedia.* Thus, in the relative size of the tentacular hair heads, in the amount of thickening of the indurated cortex cells, in the greatly reduced size of the glandular hairs of the sepals as inherited from *D. filiformis*, and in the color and size of the flowers, there is a decided preponderance in morphological detail of *D. intermedia* over the other parent, or the former exercises a certain swamping effect on the growth vigor handed down from the latter parent. This is all the more remarkable when one considers that the apparently prepotent parent is the smaller and more delicate species. Until facts can be obtained on which to base an exact explanation we can at best merely theorize. But I would again advance as a highly probable hypothesis the view given in my earlier paper, viz., that the sex cells of the pollen grain or of the ovule may have attained to a greater size in the smaller species, and may have contained a larger amount of hereditary chromatic substance. In the graft hybrid *Cytisus Adami* admirable and direct evidence is afforded of a much smaller species, *Cytisus purpureus*, having greatly larger nuclei and more chromatin apparently in its epidermal cells than the larger species, *C. Laburnum*, which has contributed with it to the formation of the graft hybrid. The graft hybrid itself closely resembles the smaller species in the size, appearance and relation to stains of its epidermal nuclei; not to mention other and more evident characters. A like condition may exist in the hybrid and parent forms of *Drosera*, even though scarcely, if at all, discernible under the microscope. It is hoped that a careful study can yet be made of this feature with the material now under cultivation.

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* Trans. Roy. Soc. Edin. Vol. XXXVII, p. 203.

The phenomenon which the writer termed bisexual hybridity receives several striking exemplifications. Where two more or less diverse growths have occurred, one on either parent, these have been shown to be reproduced not in blended fashion, but as distinct structures reduced either in size or number or both. The elongated glandular hairs on the sepals of *D. filiformis*, and the sessile two-celled glands of *D. intermedia* alike appear in the hybrid. Such a morphological pattern is frequent in hybrids whose parents are somewhat removed in systematic affinity, and suggests interesting cytological speculation. For, if every cell in the hybrid be, as its structure proclaims it to be, a combined effect of two parental conditions each reduced by half, some appropriate explanation must be given to the special case before us. As yet we have no evidence which would militate against the view, and everything is in favor of it, that every average cell of a hybrid has an equal number of chromosomes and half as much chromatic substance as is found in each parent. But for the production of two such epidermal appendages some special line of development must have been taken by the epidermal cell which gave rise to each. The view would be an imperfect one which would cause us to suppose that chromosomes representative of one parent were alone present in such epidermal cells. It will be more consonant with the principles of heredity, if we suppose that at a certain cell centre in the epidermis, a special growth-potentiality is inherited from one parent, that stimulates to the formation of a hair characteristic of it, and that while the hereditary influence of the other parent, that is devoid of such hairs, is sufficient to reduce or check back growth of the hair to at least half the size of the parental one, it fails to prevent the development of a structure peculiar to one parent alone. Neither is there any need to suppose that there is a separation or sorting out of chromatic elements in the process. Side by side on the same spirem thread of the epidermal cell which produces such a hair, elements of both parents may exist, and similarly also in each cell that contributes to the hair formation. But the decidedly reduced size, in the hybrid, of the glandular hairs inherited from *D. filiformis*, seems evidence that there is a marked prepotency of the chromatic substance of the other parent over the average. It is not too much to hope that some of these hypothetical problems may yet receive full verification.

DESCRIPTION OF PLATE XII.

Fig 1. Leaf of *Drosera filiformis.*

Fig. 2. Leaf of *Drosera hybrida.*

Fig. 3. Leaf of *Drosera intermedia.*

Fig. 4*a*. Gland cells of *Drosera filiformis;* 4*b* of *D. hybrida;* 4*c* of *D. intermedia*, all in vertical view.

Figs. 5*a*, 5*b*, 5*c*. Surface views of last.

Fig. 6*a*. Stoma of *D. filiformis;* 6*b* of *D. hybrida;* 6*c*, of *D. intermedia.*

Fig. 7*a*. Capitate glandular hair from sepal of *D. filiformis;* 7*b* do. do. of *D. hybrida.*

Statistical Information Concerning the Production of Fruits and Seeds in Certain Plants.

By John W. Harshberger, Ph. D.

DURING several summers as opportunity presented, the following statistical observations were made, in order to determine what the successful termination of the act of fertilization really was. Most botanists claim, that the association of flowers together in heads, umbels, spikes and other compact inflorescences with the attractive parts attached, renders the act of pollination more certain. The production of seeds is thereby insured and the perpetuation of the species accomplished. The regnant natural orders are those which include plants that have such mechanical and physiological devices as to insure the production of a large number of good seeds from any one plant. These facts have been widely accepted, and no doubt are true in the main, but there are facts which lead us at times to believe that in some cases the theories generally advanced are unsound.

The following statistics are presented as in part a contribution to this subject: It is to be regretted that all of the factors which influenced the growth of the plants in the open could not have been taken into account. For example, the weather materially influences seed production, the position of the plants whether growing under shade, or in the bright sunlight should have been taken into account, the insect visitors ought also to have been enumerated. Still the writer believes that the tables possess considerable value, even as a mere enumeration of the ratio of perfect to abortive seeds produced.

Arisæma triphyllum, Torr. (Indian Turnip).—The racemose fruit-clusters of thirteen plants were examined. A perfect fruit is one fully matured and fleshy, an abortive fruit is an imperfect, shriveled and seedless one.

No. of Plant.	No. of Perfect Fruits.	No. of Abortive Fruits.
1	7	51
2	6	22
3	4	60
4	10	58 red.
5	8	47 "
6	11	29
7	40	10 "
8	14	24 "
9	15	53 "
10	11	48 "
11	0	32
12	9	62
13	9	41

Rhododendron (*Azalea*) *nudiflorum*, Torr. (Pinxter-Flower).—The whole bush was examined in the enumeration of perfect and abortive fruits. The two plants were quite small although fully formed.

No. of Plant.	Perfect Fruits.	Abortive Fruits.
1	4	70
2	0	20

Cornus florida, L.(Flowering Dogwood).—Professor Sargent in his Silva (V, 67) says regarding the fruit of this plant: "The fruit ripens in October, usually only three or four drupes being developed from a head of flowers." In the table below, details are given for two trees; the first tree having 34 clusters of fruits and the second one 102 clusters, originally; each of the flower clusters from which the fruits came had four white attractive bracts.

FIRST PLANT.			SECOND PLANT.								
No. of Clusters.	Perfect.	Abortive.	No.	Perfect.	Abortive.	No.	Perfect.	Abortive.	No.	Perfect.	Abortive.
1	6	15	1	6	17	35	7	15	69	6	15
2	5	10	2	5	19	36	5	12	70	5	17
3	8	18	3	4	15	37	4	18	71	6	18
4	7	7	4	6	20	38	4	14	72	3	16
5	7	9	5	2	20	39	5	14	73	7	15
6	7	13	6	5	14	40	4	9	74	3	10
7	6	18	7	3	22	41	5	15	75	6	14
8	5	14	8	1	14	42	7	17	76	4	15
9	2	15	9	4	16	43	3	11	77	3	18
10	5	10	10	3	13	44	6	13	78	5	19
11	8	24	11	5	19	45	8	17	79	6	17
12	5	20	12	7	16	46	6	15	80	3	17
13	5	12	13	8	17	47	5	19	81	6	17
14	5	10	14	5	20	48	7	16	82	5	15
15	8	15	15	4	17	49	4	13	83	6	18
16	8	15	16	6	20	50	4	12	84	4	15
17	7	15	17	4	19	51	5	11	85	6	18
18	9	19	18	5	18	52	5	16	86	5	15
19	7	15	19	6	15	53	5	13	87	5	18
20	5	12	20	7	16	54	6	16	88	1	17
21	6	12	21	5	17	55	3	18	89	5	17
22	4	7	22	7	21	56	2	20	90	5	12
23	5	10	23	5	18	57	5	12	91	7	16
24	6	16	24	5	14	58	5	14	92	5	16
25	1	10	25	3	15	59	5	18	93	7	15
26	7	5	26	4	18	60	6	15	94	5	13
27	5	17	27	4	10	61	5	16	95	5	16
28	5	5	28	1	14	62	6	13	96	4	19
29	6	8	29	5	14	63	6	12	97	6	15
30	4	15	30	5	17	64	5	20	98	5	19
31	6	21	31	5	13	65	4	20	99	6	11
32	8	12	32	7	19	66	4	16	100	3	20
33	4	10	33	4	20	67	2	19	101	7	18
34	7	19	34	7	15	68	5	14	102	5	18

Kosteletskya virginica, Gray.—The plants for this enumeration were gathered in the salt marshes at Seaside Park, N. J. The ratio of the perfect to the abortive seeds is given.

Staphylea trifolia, L. (American Bladder-Nut).—The capsule of this plant is large, membranaceous and inflated, three-lobed and three-celled. The seeds were studied statistically in order to determine how many seeds were necessary to cause the pod

Number of Plant.	Number of Capsule.	CAPSULE NUMBER.																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Plant 1 .	5	5	3	5	3:2	1:3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
" 2 .	14	5	5	3	5	5	3	5	3:2	5	1:4	5	0:5	4	3	—	—	—	—	—	—	—	—
" 3 .	12	1:3	—	5	5	4	5	5	4:1	4	5	5	5	—	—	—	—	—	—	—	—	—	—
" 4 .	13	5	5	5	4	0:4	5	4	2:2	3	5	2:3	4:1	1p.	—	—	—	—	—	—	—	—	—
" 5 .	22	3:1	5	4:1	5	5	4	4	4	4	3:2	4	2:2	5	5	5	4	5	3:2	2	4	4	—
" 6 .	18	4	5	5	4	5	5	5	5	4:1	5	5	5	4	2p.	5	2	5	5	—	—	—	—

to enlarge to its full dimensions. It was found that the formation of one seed alone was sufficient to produce a capsule of three fully inflated locules. Thirty-five capsules were examined as to the normal number of seeds present.

Cells.	CAPSULE NUMBER.																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	1	0	1	2	1	1	2	1	1	1	0	1	1	1	0	1	1	1	1	2	0	0	0	1	1	1	1	1	1	0	1	1	0	1	1
2	0	1	1	0	0	2	1	1	0	2	1	1	1	0	1	0	0	1	0	0	0	1	1	0	0	1	1	1	0	0	1	0	1	0	0
3	0	—	0	0	0	0	0	0	0	0	3	0	0	2	1	0	1	1	0	3	1	1	0	0	0	0	0	3	0	3	0	0	1	0	0

Hibiscus Moscheutos, L.—The ripe capsules on a number of plants of this species were counted in 1894 at Seaside Park, N. J., where it grows abundantly in the salt-water marshes. The results statistically are displayed in the subjoined table (p = pierced by larvæ).

CAPSULES.

No. of Plant.	One.			Two.			Three.			Four.			Five			Six.		
	Seeds.		No. Cells.	Seeds.		No. Cells.	Seeds.		No. Cells.	Seeds.		No. Cells.	Seeds.		No. Cells.	Seeds.		No. Cells.
	Perfect.	Abortive.		Perfect.	Abortive.		Perfect.	Abortive		Perfect	Abortive		Perfect.	Abortive		Perfect.	Abortive.	
1	71	55	3	137	14	4	117	30	5	102	46	5	—	—	—	—	—	—
2	82	34	5	105	11	5	*47	64	4	82	36	5	—	—	—	—	—	—
3	68	23	5	*40	51	5	52	46	5	51	49	5	*38	57	5	—	—	—
4	113	5+12p	5	*49	57	5	—	—	—	—	—	—	—	—	—	—	—	—
5	107	15	5	88	33	5	114	12	5	78	39	5	110	10	5	110	16	5
6	*50	70	5	61	61	—	113	12	5	115	10	5	—	—	—	—	—	—
7	59	53	5	84	25	5	105	4	5	97	5	5	87	11	5	—	—	—
8	115	1	5	110	1+15p	5	46	36	5	109	14	5	—	—	—	—	—	—

The numbers marked by an asterisk show us, that as respects the number of seeds contained in any given capsule of a plant, the result of the act of fertilization has not been so successful.

Xanthium canadense, Mill.—A count was made of the fruits of a plant (*var. echinatum*) at the seashore in sand which showed five well-developed fruits, and a plant growing in gravel near Philadelphia, which had 622 fruits.

Yucca filamentosa, L. (Adam's Needle).—This liliaceous plant is of considerable interest from a biological standpoint owing to its dependence upon the moth, *Pronuba yuccasella*, Riley, which passes part of its larval existence in the capsule of the plant feeding upon the seeds. The moth, previous to depositing its eggs in the soft ovary, pollinates the stigma by placing a ball of pollen from the same flower between the three stigmatic lobes. This insures the production of good seeds on which the larvæ feed. The following table gives the number of circular holes drilled by the larvæ in leaving the capsule to descend to the ground, the number of good seeds, the number of seeds devoured by the larvæ, and the number of abortive seeds. There are two rows of seeds in each of the three locules of the capsule; a false partition present gives the impression that the capsule is six-celled. The fruit vessel is usually constricted in the middle through the irritation caused by the puncture made by the parent moth, so that it becomes more or less dumb-bell shaped. In the enumeration, the two rows of seeds in each cell are designated first side, second side. The ratio established is that of the fully formed, black seeds to the abortive seeds. By adding the seeds of normal appearance of the first two columns, lettered first and second sides, and subtracting the number of seeds devoured as well as the bad seeds of normal form not given in the table from the sum thus obtained, the figures for the last column of the table were obtained, giving the number of seeds capable of germination. Eight capsules were examined September 11, 1894, and the results tabulated, as follows:

	Number Seeds First Side.	Number Seeds Second Side.	Number Holes First Side.	Number Holes Second Side.	Seeds Devoured First Side.	Seeds Devoured Second Side.	Number Aborted Ovules in Constriction.		Number Holes in Capsule.	Number Good Perfect Seeds.
							I.	II.		
I. Capsule.										
1 Cell	36:13	28:12	—	—	—	—	—	—	5	64
2 Cell	18:11	28:15	1	2	11	15+12	—	9		8
3 Cell	31:12	25: 9	2	—	15+ 9	5 over from other.	—	—		27
										— 99
II. Capsule.										
1 Cell	24:22	23:11	—	2	9	12+ 6	3	5	7	20
2 Cell	24:16	25:18	1	1	15+ 8	6	5	3		20
3 Cell . . .	24:12	18:12	2	1	10+11	5	7	—		16
										— 56
III. Capsule.										
1 Cell	34:10	25:15	1	—	15	—	1	5	6	48
2 Cell	25:18	30:12	1	2	12+ 7	20+ 5	8	2		11
3 Cell	31: 9	30: 7	1	—	18	18	6	3		25
										— 84
IV. Capsule.										
1 Cell	26: 7	29: 8	0	1	3	17	0	5	4	32
2 Cell . . .	18:14	20:22	1	0	10	6	12	12		17
3 Cell	25:11	30:14	2	0	24	8	7	2		20
										— 69
V. Capsule.										
1 Cell	35: 8	36:10	1	1	17	15	7	7	3	30
2 Cell	14:28	22:21	0	0	0	0	22	0		34
3 Cell	28: 9	29:15	1	0	9	14	0	8		41
										—105
VI. Capsule.										
1 Cell	25:16	29:20	0	0	0	0	7	8	4	53
2 Cell	25: 7	32: 9	1	1	17	15	5	2		25
3 Cell	28:13	18:25	1	1	7	12	3	6		23
										—101
VII. Capsule.										
1 Cell	20:13	18: 7	1	1	12	14	4	3	4	6
2 Cell	22: 7	16:19	1	0	12	15	3	8		11
3 Cell	24:11	29:11	1	0	0	0	8	—		52
										— 69
VIII. Capsule.										
1 Cell	28: 8	31:11	1	1	14	14	5	—	6	23
2 Cell	27: 4	30:16	0	0	12	8	6	10		36
3 Cell	29: 6	27:14	1	0	12	10	5	10		33
										— 92

+ mark indicates that the seeds above and below the constriction are added.

Pimpinella integerrima, Benth. & Hook.—In the Umbelliferæ a perfect fruit (cremocarp) consists of two halves (mericarps). In the table below the cremocarp only is taken into consideration in the estimation of the number of perfect and abortive fruits. The plants were gathered at Hagerstown, Md., August 10, 1894, along Antietam Creek, on limestone soil with a northern, shade exposure.

	Plant I.		Plant II.		Plant III.		Plant IV.		Plant V.		Plant VI.	
	36 Umbels.		15 Umbels.		32 Umbels.		32 Umbels.		22 Umbels.		38 Umbels.	
	Perfect.	Abortive.	Perfect.	Abortive.	Perfect.	Abortive.	Perfect.	Abortive	Perfect.	Abortive	Perfect.	Abortive.
1 . . .	42	20	105	11	59	0	6	12	176	15	180	8
2 . . .	191	31	54	16	117	0	170	2	156	4	65	12
3 . . .	95	15	22	14	5	11	121	0	107	7	60	7
4 . . .	220	1	24	23	65	12	66	31	238	4	14	9
5 . . .	49	11	0	0	10	14	128	13	160	2	230	5
6 . . .	49	22	0	7	19	0	7	24	30	15	267	2
7 . . .	115	30	37	23	100	14	29	4	39	11	172	3
8 . . .	43	17	35	23	81	0	38	5	167	2	145	5
9 . . .	87	11	0	0	42	0	126	0	52	6	100	8
10 . . .	49	12	0	0	61	0	36	2	41	6	141	1
11 . . .	71	14	0	0	27	30	125	0	32	10	112	0
12 . . .	80	13	0	0	12	12	50	56	38	4	140	7
13 . . .	55	20	0	0	40	20	41	40	30	30	74	13
14 . . .	35	9	0	0	50	12	52	66	46	8	61	6
15 . . .	40	19	0	0	57	17	45	75	43	10	72	5
16 . . .	65	13	—	—	10	13	14	19	45	5	43	12
17 . . .	35	9	—	—	8	30	16	34	0	10	51	16
18 . . .	6	11	—	—	12	2	30	30	0	10	62	6
19 . . .	42	18	—	—	18	32	38	35	7	0	32	25
20 . . .	11	16	—	—	51	4	13	35	0	2	27	24
21 . . .	7	9	—	—	51	8	28	73	122	12	53	16
22 . . .	8	10	—	—	12	23	24	9	—	—	20	21
23 . . .	5	3	—	—	44	4	46	65	—	—	80	7
24 . . .	7	9	—	—	34	9	13	36	—	—	32	20
25 . . .	2	10	—	—	4	12	25	44	—	—	21	29
26 . . .	1	28	—	—	53	12	1	40	—	—	77	4
27 . . .	5	10	—	—	14	12	18	34	—	—	16	6
28 . . .	8	23	—	—	11	5	2	27	—	—	3	1
29 . . .	2	7	—	—	0	11	16	26	—	—	20	30
30 . . .	6	9	—	—	14	12	7	30	—	—	0	7
31 . . .	7	16	—	—	8	12	2	35	—	—	33	11
32 . . .	0	22	—	—	7	11	204	12	—	—	69	15
33 . . .	6	4	—	—	—	—	—	—	—	—	1	14
34 . . .	34	19	—	—	—	—	—	—	—	—	8	3
35 . . .	0	6	—	—	—	—	—	—	—	—	2	8
36 . . .	1	12	—	—	—	—	—	—	—	—	11	9
37 . .	—	—	—	—	—	—	—	—	—	—	1	6
38 . . .	—	—	—	—	—	—	—	—	—	—	9	18
Total . .	1479	509	277	117	1096	354	1537	914	1529	173	2504	399

The plants showed two distinct kinds of umbel, those at the top of the main stem and side branches which bore the largest number of perfect fruits, and the lateral umbels at the side and beneath the more prominent ones, which bore relatively a smaller number of perfect cremocarps. This suggests, that the abortive condition of many of the fruits was due to the preponderating amount of food being supplied to the favored umbels to the exclusion of the later formed and disadvantageously situated ones.

The relative ratios established in these tables between the perfect and abortive seeds and fruits give some idea as to the success of the act of pollination and fertilization. It is surprising, when we set aside our *a priori* position and draw up statistical tables, to find in a number of instances that the abortive seeds and fruits exceed the perfect ones.

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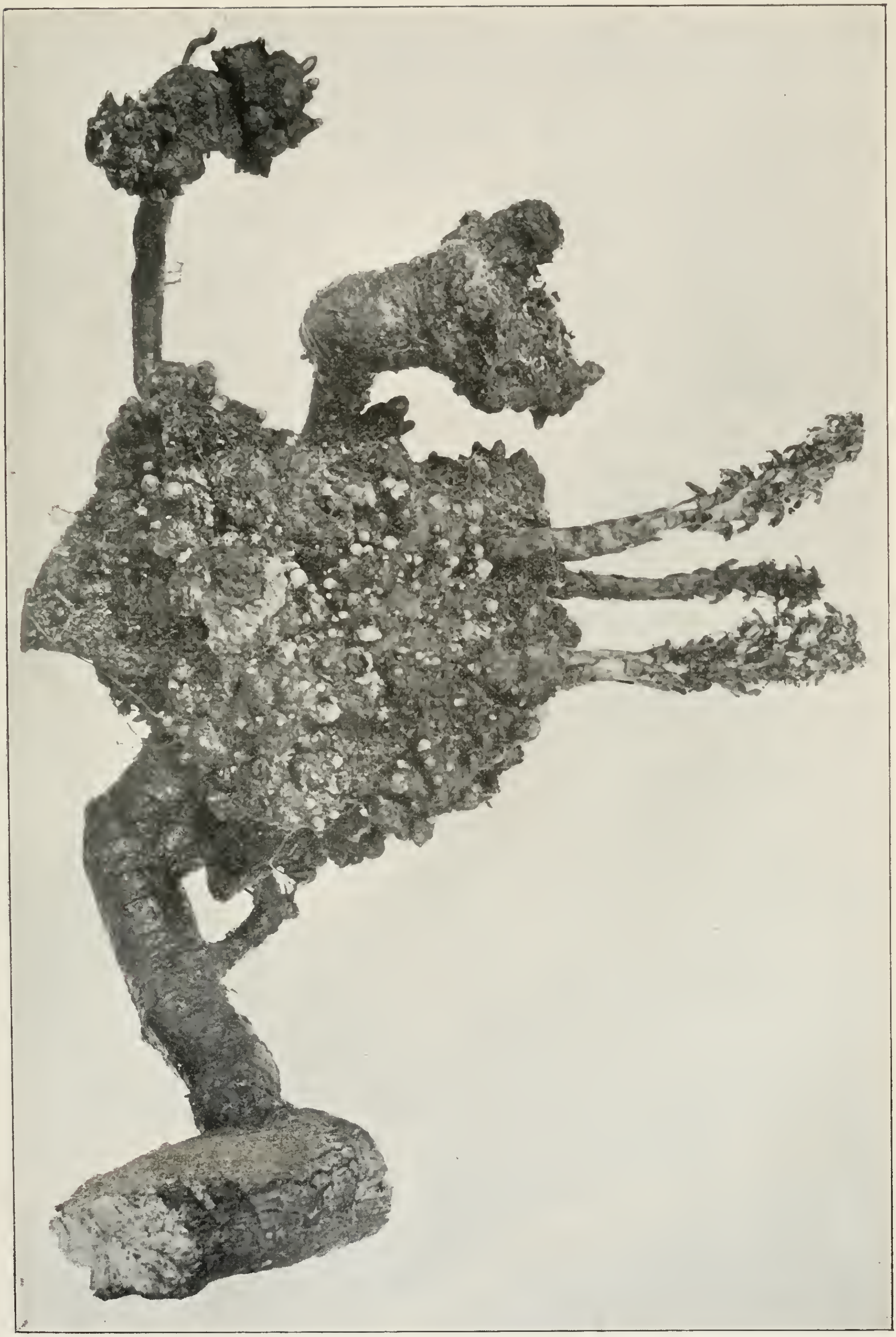

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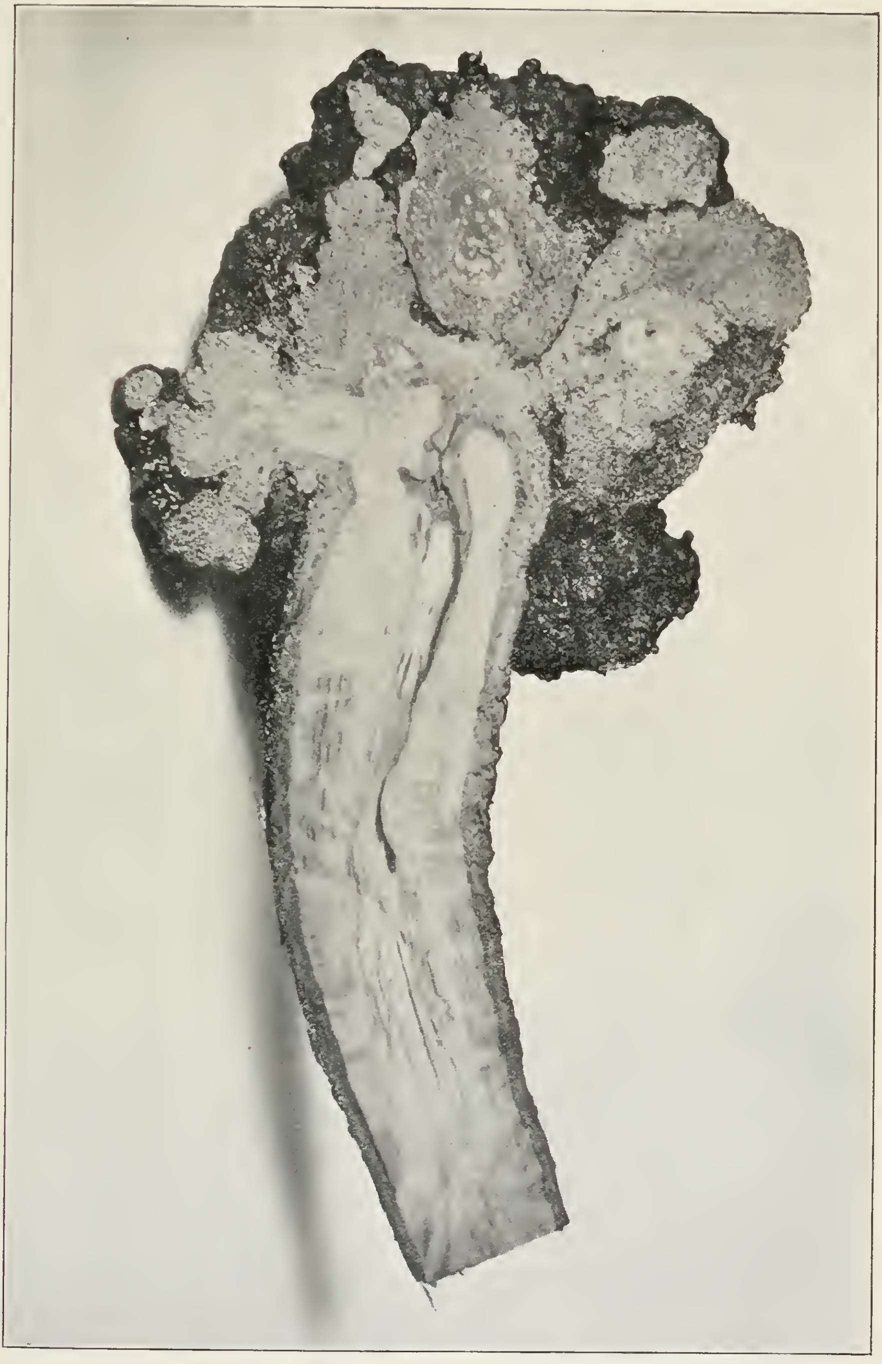

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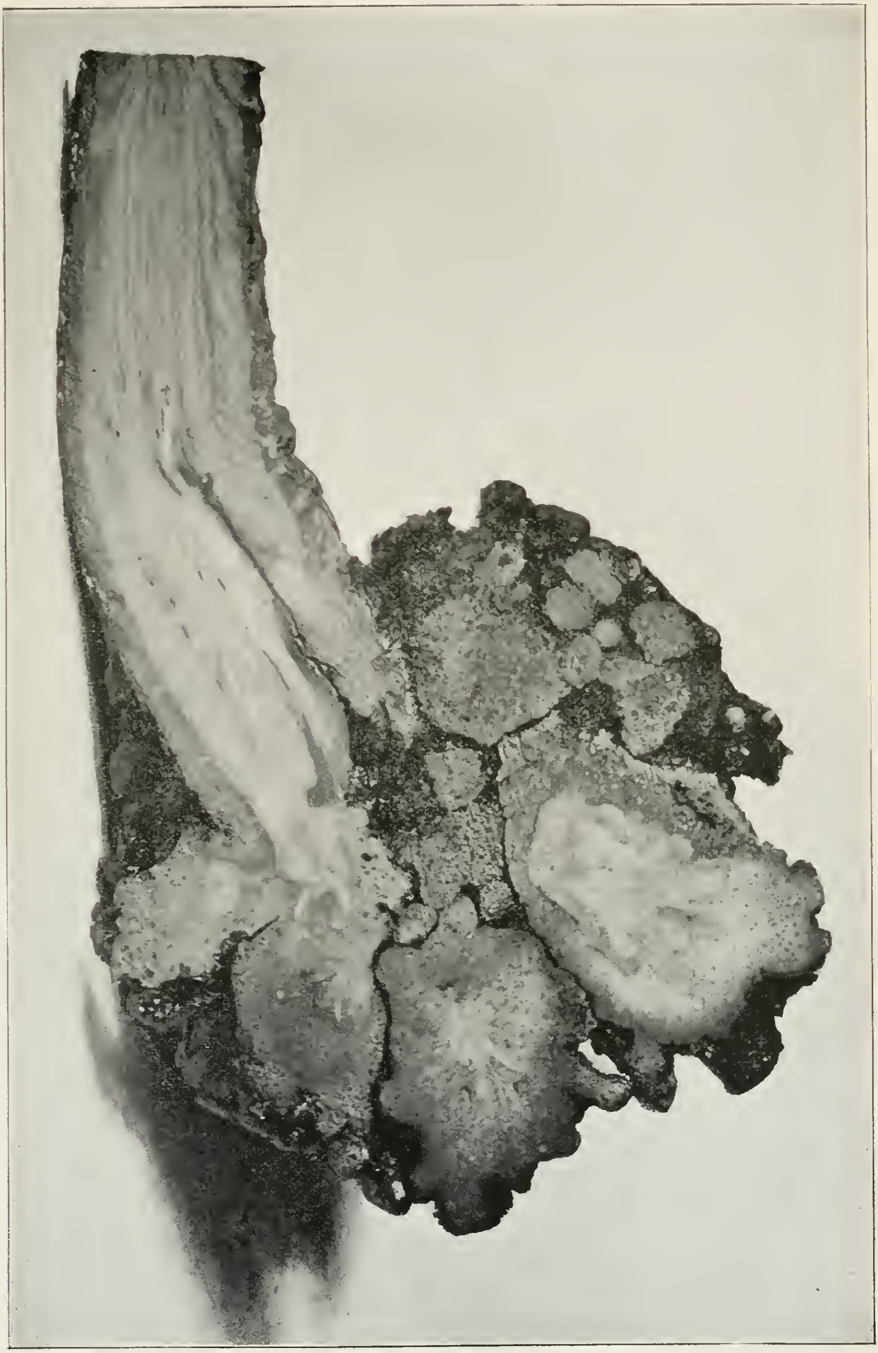

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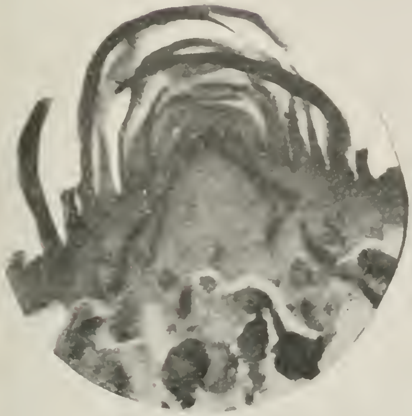


FIG. 1.

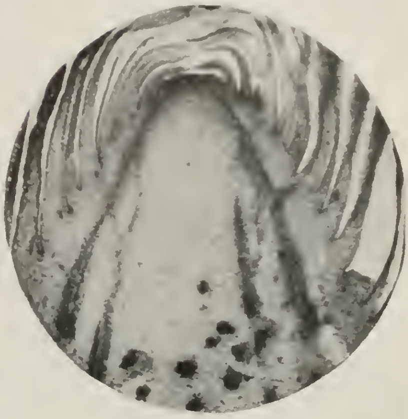


FIG. 2.

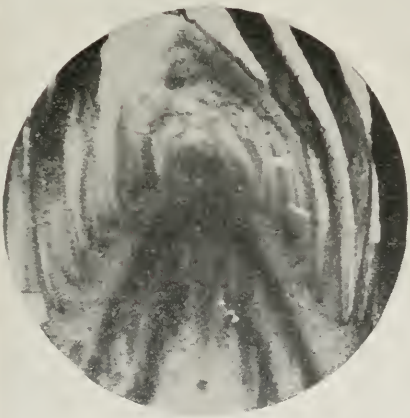


FIG. 3.

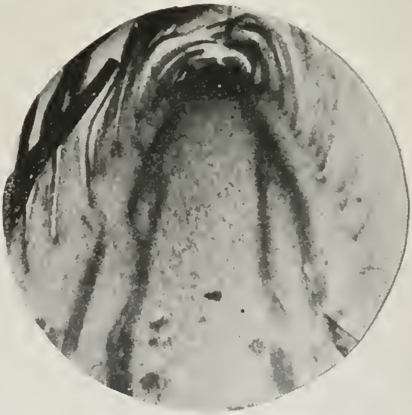


FIG. 4.

WILSON ON CONOPHOLIS.

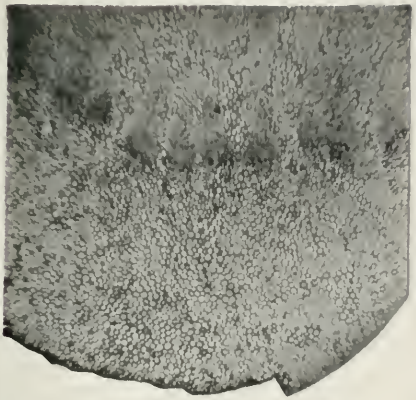
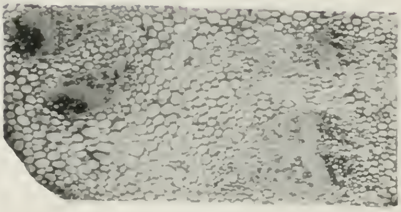


Fig 1



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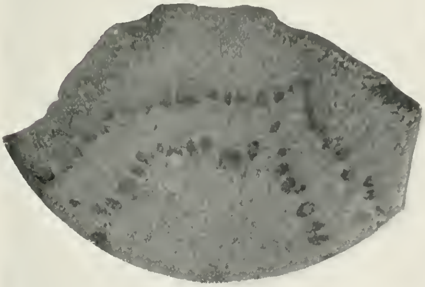

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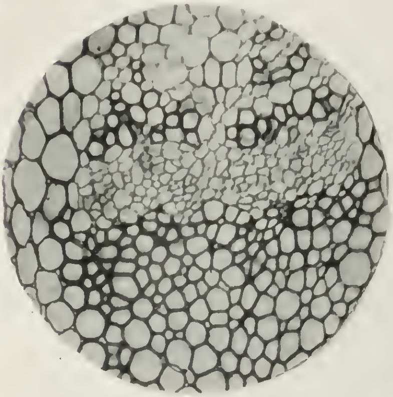

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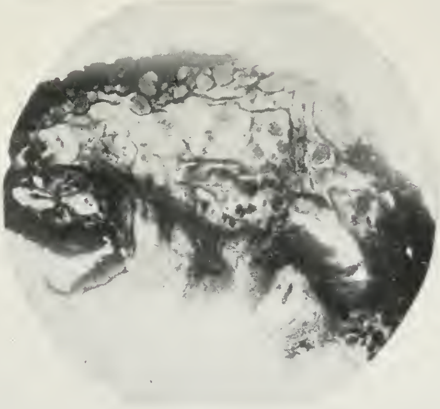
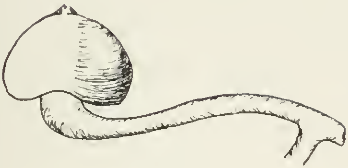


Fig 5

Wilson on Conopholis.



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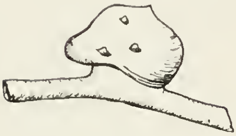

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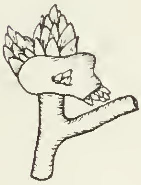
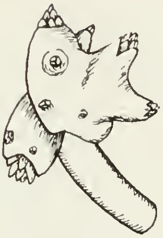


FIG 3.



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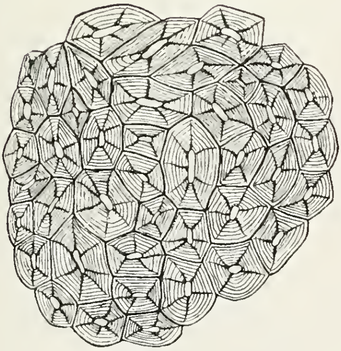


FIG. 5.

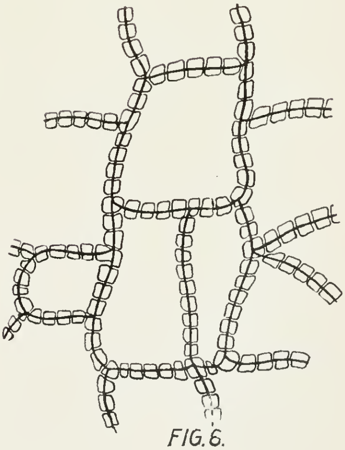
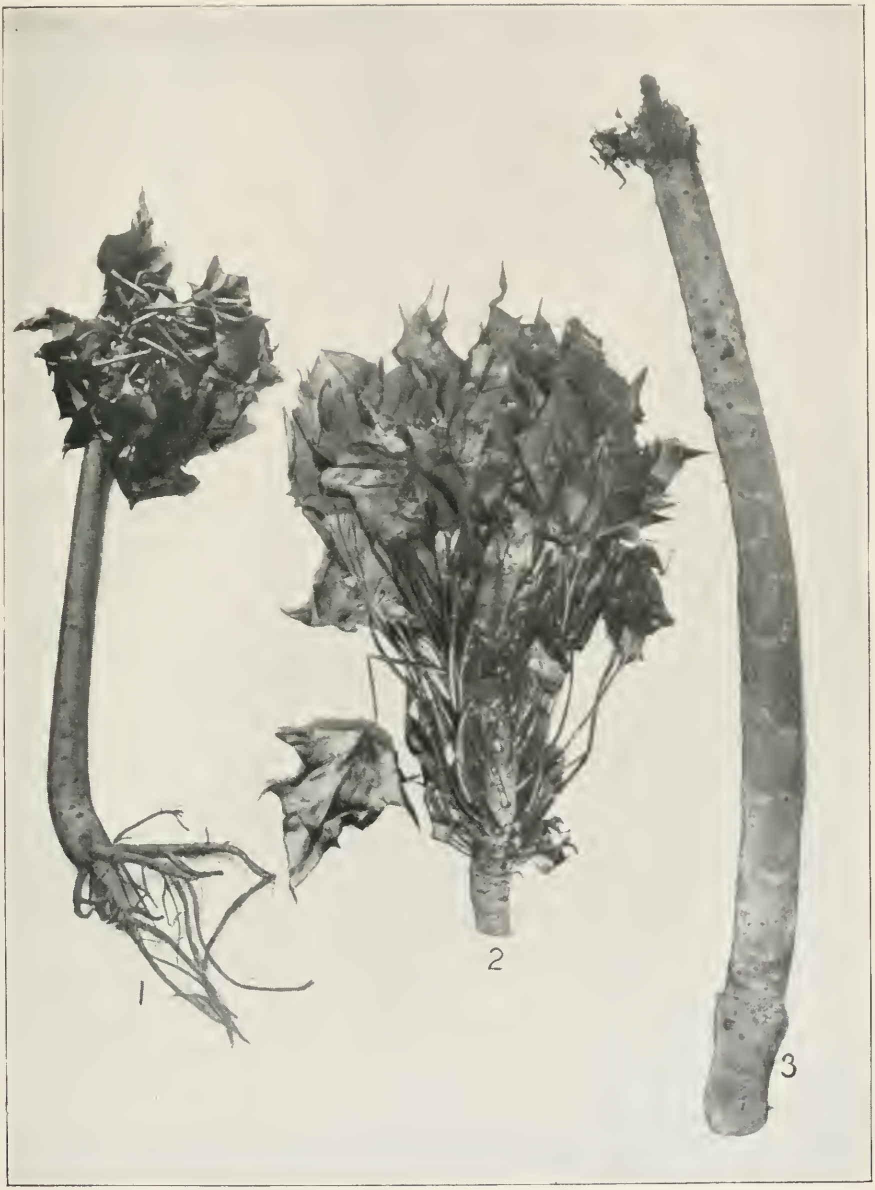
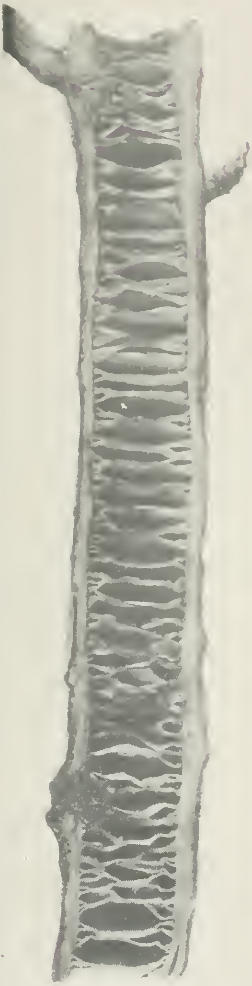


FIG. 6.

Wilson on Conopholis.



HARSHBERGER ON SENECIO PRAECOX.



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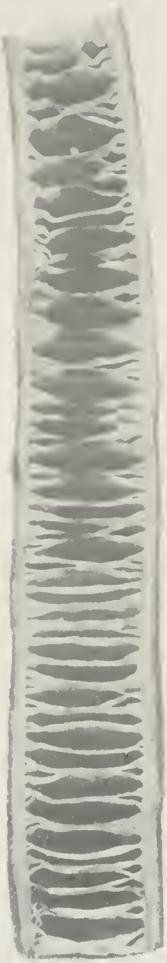

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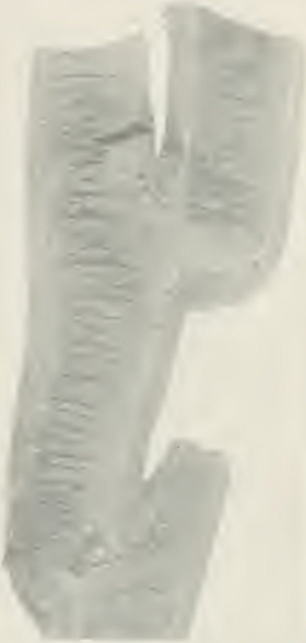

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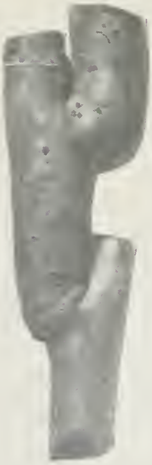
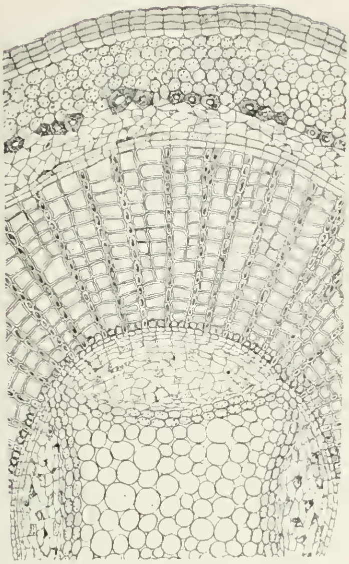


Fig. 3

Harshberger on Senecio praecox.



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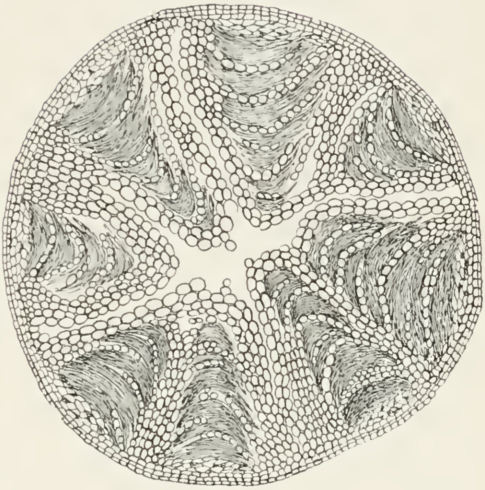

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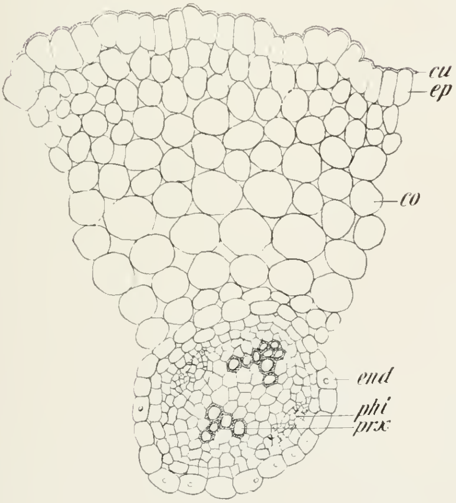
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FIG. 3.

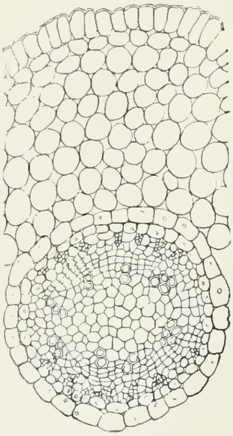


FIG. 4.

Thompson on Gelsemium.

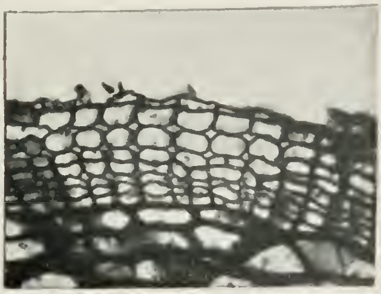
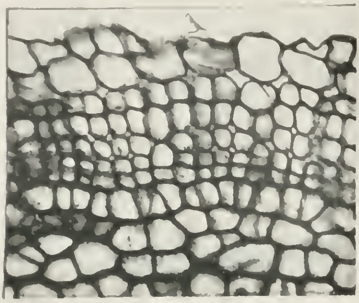


FIG. 1.



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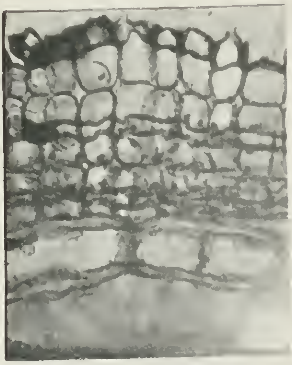


FIG. 3

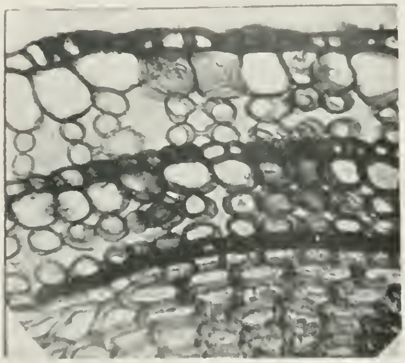


FIG. 4.

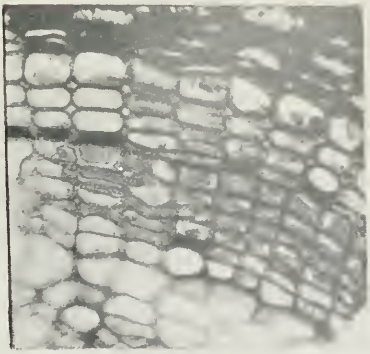


FIG. 5.

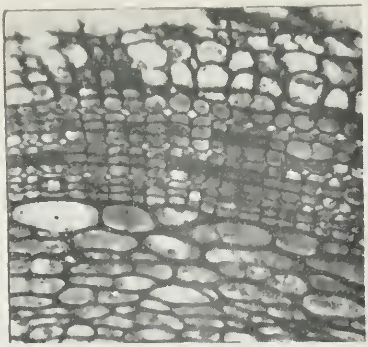
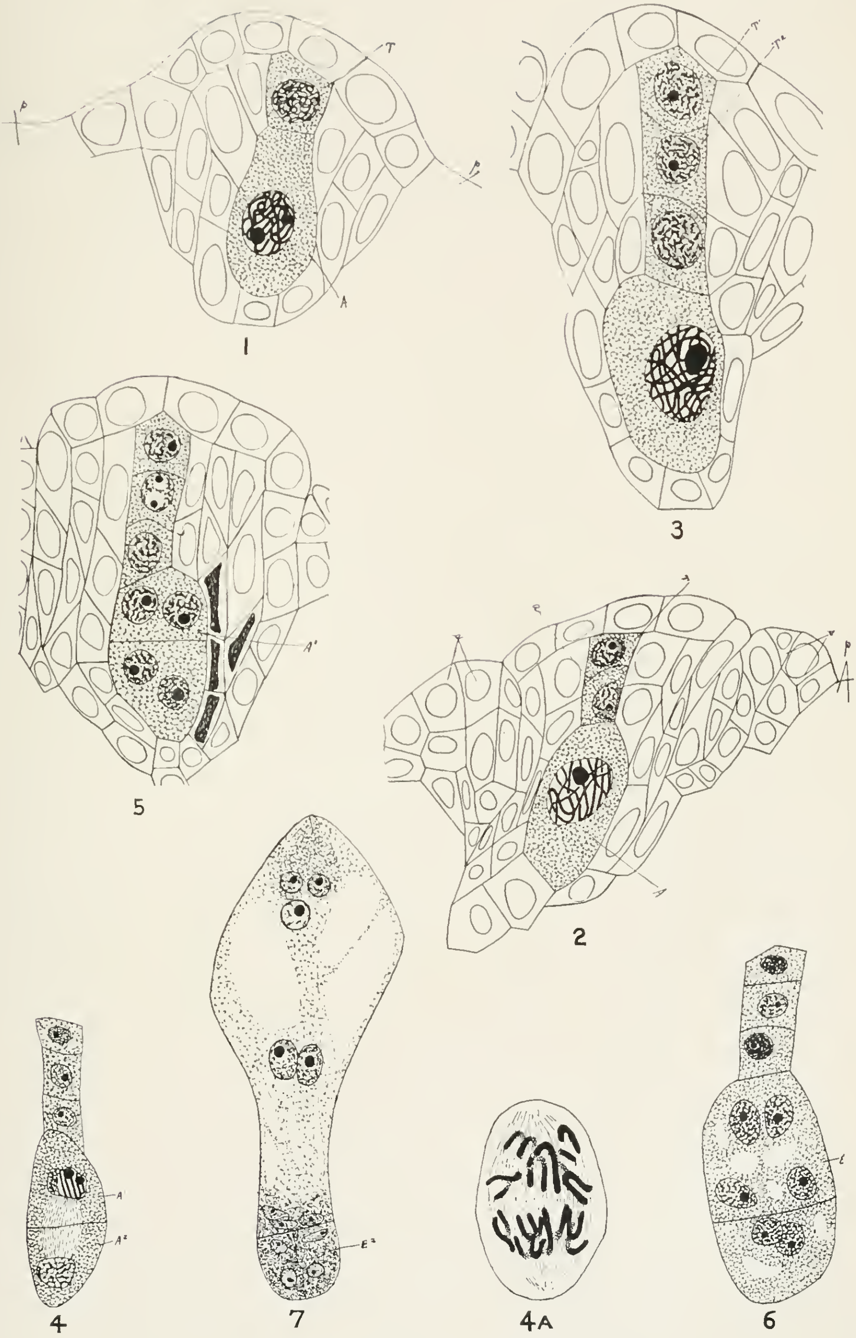


FIG. 6.

Bunting on Rosaceous Roots.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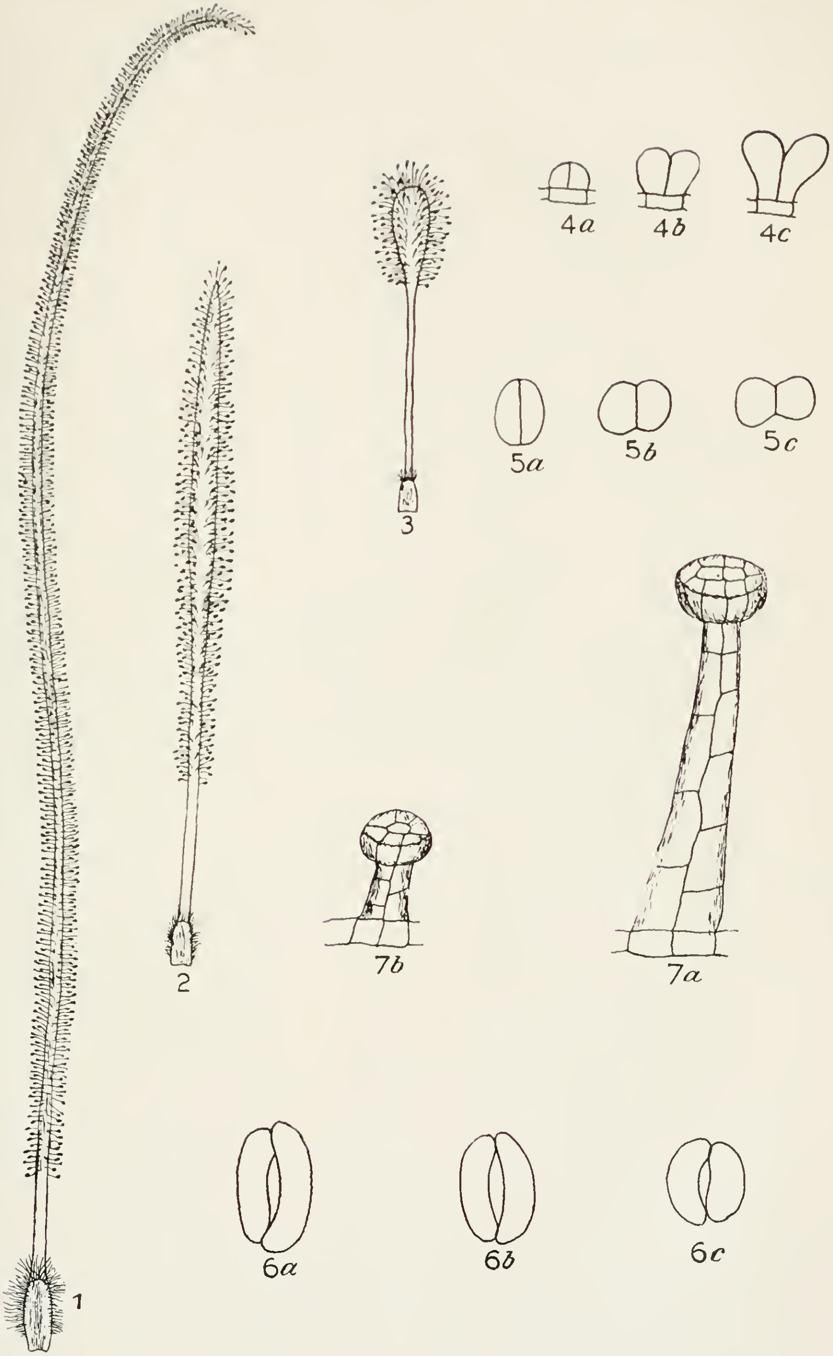
McKenney on Embryo-Sac.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7a
7b

Macfarlane on Hybrid Droseras.

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The Structure and Parasitism of Aphyllon Uniflorum, Gray.

(*WITH PLATES XIII-XV.*)

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By Amelia C. Smith, B. S.

Aphyllon uniflorum, or the Naked Broom-rape, belongs to the large parasitic order of the *Orobanchaceæ*, and is by some included in the genus *Orobanche*. It is typically a North American species, and is a pure parasite, being without chlorophyl. The present study was undertaken at the suggestion of Professor Macfarlane, and was carried out under his direction. All of my material has been collected from one very limited locality at Glenolden, Pennsylvania, where it grows luxuriantly. It appears toward the end of May, when Aster plants are about nine inches high, and by the end of June the seeds are matured and the plant is withering. I have invariably found it parasitic on the roots of *Aster corymbosum*. Chatin states that it is parasitic on *Solidago* and other Synantheræ, while Beck gives the following list of host-plants: Species of *Artemisia*, species of *Solidago*, *Sedum stenopetalum* (!) By carefully cutting out and lifting a large sod containing several Asters and a clump of *Aphyllon*, and then washing away the soil until the roots were exposed, I was able to trace the root-connections with certainty.

METHODS.

Some of my material was killed in saturated aqueous solution of corrosive sublimate, a smaller quantity in absolute alcohol, and a great deal of it was simply preserved by placing it at once in 70 per cent alcohol. For general structure, all of the above methods gave equally good results. For

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histological detail, and for embryological study, all of these methods proved equally bad. The protoplasm was somewhat shrunken even in the best specimens, and of course this was especially marked in the macrospore and surrounding cells of the ovule.

General Morphology.

The plant is of a pale purplish-white color, and attains a height of from four to five inches. The roots are thick and fleshy, arise in clusters from the base of the stem and branch quite freely in all directions. Roots forming parasitic connections either end in a knob-like sucker, or (as is more usual) may continue past the sucker and end as a soil-root. Sometimes one *Aphyllon* root forms several suckers on the same or on different Aster roots. The soil-roots are more numerous than the parasitic ones, and from the structure of the former it seems probable that they are functionless as absorbing agents, and serve merely as hold-fasts.

The stem is short, fleshy, and relatively very thick, often more than one-quarter inch in diameter, and from one to one and a half inches in length. It may branch several times at the base, or may remain unbranched. Foliage leaves are entirely absent, or else are represented only by one or two very thin scale-leaves at the base. The bracts are the conspicuous leaves of the plant, and in a stout, vigorous specimen may number five to ten. In the axils of the upper bract-leaves are developed the long, hollow scapes, each bearing a single irregular, purplish-white flower. Upper bract-leaves, scape and flower are thickly clothed with glandular hairs.

I have found no trace of any perennating structure, and the plant seems to be strictly an annual.

Structure of the Roots.

The epidermis is of flattened, somewhat thick-walled cells. Some contain globules of a peculiar oil-like substance, but it is not uniformly present, and some of the cortical cells show it also. I have found no trace of a symbiotic fungus in the epidermis.

Beneath the epidermis is the cortex, some ten or twelve cells deep. These cells contain many small, rounded starch grains, which are usually placed on the lower and inner sides of the cell. Chatin states that the epidermis contains fine granules, and the parenchyma large granules, "ni verts ni amylacés," but Koch states that starch is invariably present in the roots of *Orobanche*. Chatin may possibly have reference to the oil-like globules mentioned above.

Within the cortex is a reduced and degenerate bundle-system. The bundle-sheath is quite absent in some roots, in others it is represented in patches. The arrangement of wood and bast varies considerably. It is most commonly diarch. A modification of this is found, in which the wood projects on one side of the bundle, so that the phloem of that side is subdivided. A smaller proportion show a triarch arrangement, while in some there seems to be an indiscriminate distribution of wood elements. The xylem consists of rather short, pitted-reticulate elements, and though less in bulk than the phloem, is better differentiated. The phloem is composed of elongated elements, filled with highly granular contents, in which nuclei frequently persist. Sieve-tubes seem to be entirely absent. Protoplasmic connections between the cells corresponding functionally in all probability to similar processes in sieve-tubes were sometimes observed.

Root-hairs are either entirely absent, or are doubtfully represented by a few small, widely scattered, dermal papillæ. Absorption must therefore be impossible for these roots. This is in harmony with Koch's statement as to the total absence of root-hairs in *Orobanche speciosa*, *O. minor*, *O. ramosa*, and *O. Hederæ*, which he studied embryologically. Koch finds a root-cap on the soil-roots, *i. e.*, the secondary roots of the above-mentioned species of *Orobanche.* They do not seem to be present in *Aphyllon*, but as I have not obtained any very young stages, it is possible that the root-caps are formed here also, and later slough off.

PARASITIC CONNECTIONS.

Parasitic connection with the host is effected by large haustoria, which fasten upon the host-root. The *Aster* roots are usually less than half the diameter of the *Aphyllon* root, but are harder and more woody. As a rule, they are not shriveled beyond the point of contact, but pass through the cluster of *Aphyllon* roots and enter the soil beyond them. The host-root is not completely surrounded by the sucker, but usually remains distinct and quite unmodified in structure on the side away from the parasite. The sucker is covered with an epidermal layer similar to that over ordinary *Aphyllon* roots, and this is quite continuous with the epidermis of the host-root. Beneath the epidermis the haustoria are composed of parenchymatous tissue, with strands of bundle-tissue, which branch almost at right angles from the bundle-tissue of the main root. This parenchyma spreads and mingles with the parenchyma of the host so intimately that it is impossible to distinguish their boundaries (Plate XIV, Fig. 2). The xylem and phloem elements of the parasite pass into the xylem and phloem regions of the host, and mingle with the corresponding elements of the host-bundle. The general position of the bundle of the host, is little modified however, and there seems to be no separation and isolation of bundle elements from the host-root within the tissue of the parasitic tubercle, such as occurs in *Conopholis.*[1]

---

[1] L. L. W. Wilson, Observations on *Conopholis Americana*, Bot. Contrib. Univ. Penn., vol. ii, p. 12.

STRUCTURE OF THE STEM.

In cross section the stem shows an epidermis of small flattened cells, thick-walled on the outer side (Plate XIV, Fig. 3). Beneath is the cortex, composed of large, rounded cells; there are usually from eight to ten layers of these cells, although the stems vary considerably in this respect. The cortical cells are closely packed with large rounded starch grains. Within is a more or less continuous ring of degenerate bundles, widely separated in some places by medullary rays. Within the bundle-ring again is the pith, its large rounded cells packed with starch grains.

The bundles are arranged in the usual manner, with external phloem and internal xylem. A reduced bundle-sheath is present as a frequently interrupted ring of small rounded cells. The phloem consists of elongated elements with granular contents, which are sometimes nucleated. As in the root, sieve-tubes seem to be entirely absent. The protoxylem consists of one or two spiral tracheæ with cells. The secondary xylem consists of short pitted-reticulate tracheæ, strongly indurated. A cambium is not generally present, although some stems show interrupted lines of small cells which may doubtfully be interpreted as cambium. Although the xylem elements are fairly numerous in root and stem, it seems probable that the wood has nothing to do with the conduction of nutritive liquids, but serves solely to support and strengthen the plant.

The presence of such quantities of starch in a colorless parasitic plant is somewhat perplexing. The starch cannot have been brought over from the host as such, since starch is insoluble, so that leucoplasts must be present, and in considerable numbers. They are, however, small and difficult to make out clearly. Moreover it is puzzling to see such an amount of reserve food stored in all parts of a purely annual plant. Koch states that the large amount of starch stored up in a young plant immediately after germination, is to insure it against starvation in case the host perishes. But starch is present in great quantities when the seeds are nearly or quite ripe, and it is passed into the soil with decay of the plant. It can scarcely be wholly to insure a supply for the endosperm, for the amount stored there is infinitesimal compared to that in the entire plant. Further, the conditions of germination preclude the possibility that the starch is present in order that the seeds may find a rich nidus in the decaying parent plant.

BRACT-LEAVES.

The bract-leaves are thick at the base, gradually thin out at the top, and are closely appressed to the stem. The epidermal cells are small and flattened. The mesophyll is packed with starch, and is supported by a few strands of bundle-tissue, which enters the bract as one strand, and quickly subdivides. The tips of these leaves are very slightly trifid, a fact that perhaps points to a three-lobed ancestral leaf. The lower bract-leaves differ markedly from the upper. The lower ones have neither stomata nor hairs. The upper ones have numerous stomata on the free outer (morphologically under) surface, and the tips and outer surfaces are clothed with multicellular, capitate hairs. Between these two extremes, selecting a plant with five leaves, there are transitional stages.

THE FLOWER.

The flower is irregular, produces a five-parted calyx, a five-parted bilabiate corolla, four epipetalous stamens, and a superior bicarpellate ovary. Bracteoles are absent. The calyx is almost or quite regular. Its lobes are fleshy and bear hairs on the outer surface. The epidermis consists of irregular cells of wavy outline, and is pierced with many stomata. The bundle-strands enter each lobe singly, but immediately divide into three main branches.

The upper lip of the tubular corolla consists of two somewhat recurved lobes, the lower of three lobes. Each lobe is supported by two main strands of bundle-tissue, which enter separately as branches of the bundle-ring in the floor of the flower. The outer surface of the corolla bears many hairs above the line where the calyx lies against it, the inner surface not nearly so many. These hairs are all of the same type, having a stalk of several cells placed end to end, and a round head formed of (usually) eight radially arranged cells. The four epipetalous stamens are didynamous, the posterior pair being the shorter. The anther-lobes are formed in the usual way, having at first four and later two loculi. The microspores are small and spherical.

The unilocular ovary consists of antero-posterior carpels. Externally it is quadrangular in shape, corresponding to the positions of the four placentas, and the style bends forward so that the broad bilobed stigma lies at the anterior part of the mouth of the flower-tube. At the base of the anterior carpel is a large nectary sunk into the tissues of the carpel (Plate XV, Fig. 7). In cross section the ovary shows an epidermal layer of high columnar cells, the outer walls of which are thickened. Within are several (four or five) layers of rounded cells. The four parietal placentas are cushion-like ingrowths of similar rounded cells, the entire ovarian wall being packed with starch. A well-marked bundle-strand is present in the middle of each carpel, the position of the bundle being indicated externally by anterior and posterior grooves on the surface of the ovary. The small anatropous ovules are produced in great numbers (Plate XIV, Fig. 4).

The nectary shows in cross section seven to nine layers of gland cells, which are readily distinguishable by their small size, rounded outline, the absence of starch, their granular, rather deeply-stained protoplasm, and large conspicuous nuclei. In the middle of the nectary, these cells extend quite to the bundle-ring, but on each side there is a rapid transition to the parenchyma of the carpellary walls. There is no special covering of epidermal cells over the nectary (Plate XV, Fig. 7). Externally, the gland appears as a small, rounded whitish swelling at the base of the ovary. The presence of this gland seems to have been overlooked by Chatin, for he characterizes the genus as one in which the ovary is unaccompanied by an hypogynous gland.

STRUCTURE AND DEVELOPMENT OF THE OVULE AND SEED.

The mature seed, which is very small and light, is surrounded by a tough, leathery coat, whose flattened cells have thickened indurated walls. The seed itself consists of a mass of endosperm cells, packed with starch, and enclosing a small primitive embryo whose cells contain little or no starch. The embryo is undifferentiated into plumule, cotyledons or radicle. These seeds will not germinate in water, nor in a nutrient solution made from the bruised roots of the host. Koch states absolutely that the seeds of *O. speciosa*, *O. ramosa*, *O. minor*, *O. Hederæ* must come in contact with the host-root if they are to germinate, and Meehan finds the same for *Aphyllon*.

I have not yet worked out entirely the development of this seed from the young ovule, and between Figs. 2 and 3 (Plate XV), I have as yet no certain connecting links. Fig. 1 shows the first appearance of the macrospore-mother-cell in very young ovules. The ovule is here still orthotropous, and is a small conical upgrowth, covered with one layer of cubical cells and containing a large macrospore-mother-cell. Fig. 2, shows an older ovule. The ovule has here turned through an angle of nearly 90 degrees. The macrospore-mother-cell has greatly elongated, and is surrounded by a clearly-marked nucellus, while the primine has grown nearly around the nucellus. Up to this stage there has been clearly no formation of tapetal cells, and (although my material was too badly shrunken to be very satisfactory) I have reason to doubt their formation at any time. I have observed many completely anatropous ovules, which contained within the three cell layers one much elongated, darkly-staining mass of protoplasm. Many of these showed two nuclei, but a clear unmistakable transverse wall could not be observed. Koch, in his "Entwicklungsgeschichte der Orobanchen," figures for *O. speciosa* the division of the macrospore-mother-cell into four, of which the upper one is the macrospore; and this division occurs when the ovule is turned half-way. If the macrospore-mother-cell does divide in *Aphyllon*, the divisions must occur at a much later period than they do in the related species described by Koch.

Plate XV, Fig. 3, shows a much older ovule. The integuments fully surround the nucellus, and the outer one is closely packed with starch, while the nucellus is pressed against by the structures within. Owing to the lack of closely preceding stages this ovule cannot be interpreted with complete certainty as yet. The probable interpretation, based upon the development of related plants, is as follows: The multicellular cell-body represents the mass of precociously developed endosperm, which has grown up around the egg-cell into a neck-like structure. The central cell is the egg, still unsegmented, and the long, plug-like body filling the apparent neck, is the suspensor, consisting of two cells. At the opposite end of the ovule, one cell represents the remnant of the antipodal cells, which have become shrunken. Such formation of precocious endosperm is common throughout the *Orobanchaceæ*, and in *Aphyllon* it seems to take place to a greater degree than in *O. speciosa*, as described by Koch, where the neck-like upgrowth of the endosperm around the suspensor is much less perfectly developed than in *Aphyllon*.

Plate XV, Fig. 4, shows the stage immediately following. The egg has divided longitudinally, and the suspensor consists of four cells. The lowest of these is destined to become the hypophysis of the future embryo.

In Fig. 5 the egg has divided into octants, and the hypophysis is plainly marked. The suspensor is still four-celled. The endosperm has increased greatly in bulk, and numerous small starch grains are found in its cells.

Fig. 6, of Plate XV, illustrates an almost mature seed. The integuments have become thin and flattened, and the endosperm contains much starch. The embryo has attained its full development. The regions are not clearly marked out, but the hypophysis here shows only anticlinal divisions. This is in harmony with Koch's statement that the primary root forms no root-cap.

SUMMARY.

1. *Aphyllon uniflorum* is parasitic on *Aster corymbosum*. The degeneration attendant upon its parasitic habit is expressed by:

   (*a*) Absence of chlorophyl.
   (*b*) Degeneration of bract-leaves.
   (*c*) Loss of root-hairs.
   (*d*) Reduction of the bundle-system, and the greater relative development of phloem than of xylem.
   (*e*) Small size of seed and primitive embryo, and the development of this embryo within a mass of precocious endosperm which completely surrounds the embryo and suspensor.

2. Parasitic roots form intimate connections with host-roots, but the host-roots are not entirely starved beyond the point of attachment.

3. Stomata are present on bract-leaves, flower-stalk, calyx and corolla.

4. A well-developed ovarian nectar-gland is present.

5. Starch is present in great quantities in roots, stems, leaves and carpellary tissue.

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EXPLANATION OF PLATES XIII, XIV AND XV.

Plate XIII, Plant of *Aphyllon* growing on *Aster corymbosum.*

Plate XIV, Fig. 1. L. S. Root of *Aphyllon.*

Fig. 2. T. S. Root of *Aphyllon*, forming parasitic connection with root-tissues of *Aster.*

Fig. 3. T. S. Stem of *Aphyllon.*

Fig. 4. T. S. Ovary of *Aphyllon*, showing placental tissue with ovules.

Plate XV, Figs. 1, 2. Developing ovules of *Aphyllon.*

Fig. 3. Mature ovule, containing precocious endosperm and egg cell.

Figs. 4, 5. Upper part of endosperm surrounding segmenting egg cell.

Fig. 6. Macrospore enclosing endosperm and multicellular embryo.

Fig. 7. T. S. Nectary.

The Comparative Structure of the Flowers in Polygala polygama and P. pauciflora, with a Review of Cleistogamy.

(*WITH PLATES XVI-XVII.*)

BY CHARLES HUGH SHAW, PH. D.,
Professor of Botany in Temple College.

[Submitted to the University of Pennsylvania in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy.]

In the genus *Polygala* only two species, so far as known, exhibit cleistogamy. Both are natives of the Eastern United States. One is *P. polygama*, often abundant along our sandy coasts, the other is *P. paucifolia*, the beautiful so-called Flowering Wintergreen of more interior districts.

The former, alike from the abundance of the cleistogamic flowers it produces, and the presence of intermediate types now for the first time described, has been the chief subject of the present study and will be first dealt with.

The detailed description of the various types of flower may best be prefaced by recapitulating what is known of the flowers of the genus.

Conspicuous blooms are borne by all members of the genus. These are generally in racemose clusters, and are sometimes very showy. The typical aërial flower is very irregular. In the calyx the two lateral, interior sepals are greatly developed as petaloid wings. The corolla consists of three petals, one anterior and two posterior, the two lateral of the theoretical five being suppressed. Of the three the anterior one is greatly developed as a hood covering the stamens and pistil. The stamens are eight in number, the anterior and posterior ones of the theoretical ten being wanting. They are monadelphous, being united below, and also with the three petals. The anthers dehisce by pores, or more strictly, after Chodat, by short slits. The microspores are ellipsoidal, flattish at the ends and traversed by several meridional thickenings, and an equatorial furrow, at some point of which the pollen tube emerges.

The pistil is composed of two fused carpels, forming a two-celled ovary, with axile or central placenta. The style and stigma present various forms, and the two carpels are often differentiated in this region ; that is, the tip of one is represented by a functional stigma, while that of the other is represented by a variously shaped appendage.

Each loculus contains a single anatropous ovule. The seeds are frequently hairy and arillate. The embryo is fleshy and embedded in endosperm.

POLYGALA POLYGAMA.

The first mention of this plant is its description by Walter in "Flora Caroliniana," as follows :

P. polygama floris corollatis, in spicis terminalibus, apetalis in spicis fecundis paulum sub terram pratensis.[1]

It is mentioned in De Candolle's "Prodromus," and is described in various Floras with little additional information. Most writers on the subject speak of the cleistogamic flowers as apetalous. Details serving for its identification, and the fact that cleistogamous flowers are borne on subterranean branches are given in the manuals of Wood and Gray. A similar description, together with a figure, may be found in the more recent Flora of Britton and Brown.

In none of the above works is any mention made of the peculiar type of flower described in the present paper.

References to *P. polygama* in literature are scanty. Dr. Britton, Mr. Redfield and others mention it among other plants in reports of botanical excursions. Mr. Hollick[1] noticed on Montauk Point a form peculiar in color of flowers. Chodat[2] describes briefly the structure of the cleistogamic flowers, and states that they may sometimes be seen in the upper clusters.

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[1] Flora Caroliniana, 1788.

Huth,[3] in discussing different forms of fruits on plants of the same species, mentions *P. polygama* as peculiar among plants which bear cleistogamic flowers in the fact that the fruits are alike.

In the summer of 1897 Dr. Macfarlane called my attention to the fact that the plants showed certain striking peculiarities which had not yet been described, and at his suggestion I undertook an investigation of them. A reference to this work appears in Dr. Schively's paper on *Amphicarpæa.*[4] An abstract of part of my work was read at the meeting of the "Society for Plant Morphology and Physiology," held in New York City in December, 1898.

Polygala polygama grows in sandy soil in many localities along the eastern coast-line and the Gulf States, but so far as I can learn, reaches its greatest development on the islands of Martha's Vineyard and Nantucket, and the adjacent portion of the Massachusetts coast. On the islands it is a constant feature of the meadows, growing as has already been noticed (Redfield), in company with *Arctostaphylos.* etc. In the neighborhood of Wood's Holl it is abundant in all the open, dry, sandy fields, and even in woodlands; its long racemes being conspicuous among the wild flowers of the neighborhood.

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[1] Torrey Bulletin, xviii, pp. 255–56.

[2] Monographia Polygalacearum. Memoires de la soc. de phys. et d'hist. nat. de Genève, 1891, p. 135.

[3] Ueber geokarpe, amphicarpe und heterocarpe Pflanze. Abh. Ver. Naturwiss., Frankfort a. Oder, viii, 1890, p. 89.

[4] Bot. Contrib. Univ. Penn., vol. i, p. 335.

Now, whereas the plant is described and figured as having simply two types of flower—conspicuous pink-purple ones borne in nearly upright racemes, and minute, subterranean cleistogamic ones, it bears later, on aërial shoots, an abundant crop of green cleistogamic flowers, that are larger than the underground ones. The branches bearing these tend to turn downward; so that a plant in August shows many, perhaps nearly all of the shoots becoming positively geotropic, and the foliage is mingled with masses of green cleistogamic flowers (Plate XVI).

We may now consider the various types of flower found in this species, and later study the structural details of each.

(1) THE CHASMOGAMIC OR CONSPICUOUS FLOWERS.

These develop in long terminal racemes, are pink-purple in color, and so are very noticeable among the low, flowering herbs of summer. At Wood's Holl they appear first about June 20, and continue to bloom in favorable seasons till about the middle of August. The petaloid sepals are persistent, and give to the maturing fruits at a distance the impression of being still in flower. However, only two, or at most three, flowers of each raceme are actually expanded at one time. By marking flowers of many plants with threads it was found that a new bud generally opens each day.

Large numbers of them fail to set seed, so that later in the season one finds to a large extent only the naked axes of these racemes, or rows of seedless capsules.

When seeds do mature properly from them, the length of time required for full maturation is about five weeks.

(2) THE SUBTERRANEAN CLEISTOGAMIC FLOWERS.

At the same time that the first pink buds of the chasmogamic flowers become visible, one may find by digging, numerous pale branches arising from the base of the stem·and growing beneath the soil. In the axils of their small bracts are white or pinkish protuberances, the subterranean flowers. At the time of pollination they are about one millimeter in length, but after pollination they rapidly enlarge, and in a surprisingly brief period assume the proportions of seed capsules. Thus, although such flowers appear in the Wood's Holl neighborhood from the fifteenth to the twentieth of June, about the same time with evident flowers, they ripen seed sooner. As early as July 4 seeds from these cleistogamic flowers may be found. Seeds are also produced much more abundantly from these than from the chasmogamic flowers.

(3) Aerial Cleistogamic or Intermediate Flowers.

Mention has already been made of the fact that another and unrecognized type of flower exists in this plant. In July, after both the evident and the underground cleistogamic flowers have been developing for a time, aërial branches may be seen bending downward. These bear, in addition to small leaves, little green tubercular bodies which are, in fact, another set of microscopic flowers. They are produced in such abundance, too, that small though they are, it is remarkable that they have never been clearly recognized and described.

They are considerably larger than the underground type and bright green, while all parts of the flower are more developed. Their development is generally associated with geotropic tendencies on the part of the shoot bearing them. It is especially striking to note that a leafy shoot, after having grown upwards and perhaps borne evident flowers, may begin to develop these small closed ones, and in so doing to bend over at its tip to pursue the opposite course. At Wood's Holl experiments were tried of digging up sods of plants bearing geotropic shoots and inverting them. In a day or two the tips were seen to show reversed curvature, thus reasserting their geotropic tendencies.

These aërial cleistogamic flowers bring forth fruit with the same rapidity as do the underground ones, and unlike those of the chasmogamic, their fruits generally mature properly.

It may be here stated in advance that the structural characteristics of these green cleistogamic flowers are strikingly intermediate between those of the other two types. Every transition stage from one to the other has been obtained. Series of forms connecting the green and underground cleistogamics are easy to find. On the other hand, it is not infrequent to find transition types between the green cleistogamics and those having the fully developed perianth. In this respect our plant calls to mind very strikingly the series of transitions already described for *Amphicarpæa* by Dr. Schively.

COMPARATIVE MORPHOLOGY OF THE CHASMOGAMIC, AERIAL CLEISTOGAMIC AND SUBTERRANEAN CLEISTOGAMIC FLOWERS.

We will now pass to a detailed consideration of the morphology of the various types of flower. This may best be done by taking up points of structure successively, and comparing them in the three forms of flower.

Inflorescence Axis.— In order to describe the rachis we refer for a moment to the structure of the vegetative stem. This has, exteriorly, several winglike angles. The wood appears at an early period as a zone. In an ordinary shoot it is difficult to find traces of separate vascular bundles, since the wood and bast early become continuous rings. The phloem ring is provided with numerous indurated cells. Without is a layer of cortical parenchym six or eight cells thick, limited by a strong cuticle. Incidentally it was noticed that for some reason, when stem sections were treated with iodine, the woody portion presently showed a strong red coloration. This seemed to suggest the presence of sugar. The reaction took place in various specimens, differently preserved.

The axis bearing the evident flowers is rather sharply three-angled and slightly winged. In the phloem the indurated layer forms a nearly continuous ring.

The axis bearing the aërial cleistogamic flowers is smaller in diameter and faintly three-angled, two angles being more or less winged. The induration of the phloem is not so great.

The axis bearing the subterranean cleistogamic flowers is variable in size, but averages considerably larger than the preceding. It is elliptical in section, and there is scarcely any induration in the phloem. The cortical parenchym is very loose and spongy, and the external cuticle only faintly formed. Sometimes the medulla is almost obliterated, and the wood then appears as a central strand.

Bracts.—Small bracts are present in the evident raceme, but they fall away about the time of flowering. In the racemes of both sorts of cleistogamic flowers, they are persistent as small scales.

Calyx.—A. The Chasmogamic Flower. In this the three outer sepals are small, green, 1½–2 millimeters long, ⅔–1 millimeter wide. The two inner sepals or wings are large, pink-purple, and projecting beyond the corolla and stamens enclose these inner members. The posterior one is slightly larger. Referring for a moment to the foliage leaf, its epidermis is composed of cells with wavy outlines and peculiarly thickened walls, as shown in Plate XVII, Fig 10. Stomata are found over both surfaces. In comparison with the foliage leaf both upper and lower epidermis of the three outer sepals consist of wavy-walled cells, rather like those of the lower leaf epidermis, but more elongated. The walls, perpendicular to the surface, have not the peculiar, inwardly projecting thickenings of the former. The outer epidermis is well supplied with stomata, especially in the middle region. On the inner surface the stomata are rare.

Chodat,[1] in speaking of the two winglike sepals in the *Polygalaceæ* says: "The anatomical structure of these wings is that of the petals. Stomata have never been found on them." The following description will show the incorrectness of the above statement. The cells of each surface are wavy in outline, similar to the smaller sepals (Fig. 11). On the outer surface stomata are numerous and on the inner they are also tolerably abundant. In both cases they occur in the central portion of the member, but cease abruptly toward the margins, where the fibro-vascular bundles end. Thus, taking into account the loose and spreading character of the wings, it will be seen that over the calyx stomata are found on surfaces which obtain free access of air, and rather irrespective of the presence of chlorophyll.

Calyx.—B. The Aërial Cleistogamic Flower. In this all five sepals are present, but the wings are reduced and only slightly larger than the other three. The epidermal cells show to a less degree the wavy outlines of the corresponding cells in the conspicuous flowers (Fig. 12). The superficial wall-thickenings are not visible. On the outer surfaces of the three exterior sepals stomata are *exceedingly abundant.*

Calyx.—C. The Subterranean Cleistogamic Flower. In this five sepals are still present. The wings are greatly reduced and are slightly smaller than the other three sepals. The wavy cell-walls, found in the epidermis of the chasmogamic flower sepals, are here lacking (Fig. 13). Stomata are present on all five sepals over both surfaces, though they are few in number, and are of a characteristically degenerate appearance.

Corolla.—A. The Chasmogamic Flower. In it the anterior petal is 5-6 millimeters long, and is developed into a hood, covering the sporophylls and bearing on the anterior border of the hood six or eight finger-shaped appendages, which project as a feathery tuft when the flower is open. The two posterior petals are about the same length, but are much narrower, lanceolate, and fused below with the hooded petal and stamen. The petals are composed of elongated, spongy, thin-walled cells, and stomata have never been found on them.

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[1] Chodat, *loc. cit.*

Corolla.—B. The Aërial Cleistogamic Flower. The anterior petals are tolerably well developed, nearly as long as the sepals and more or less fashioned into a hood, partly enclosing the stamens and style. The posterior petals, rarely wanting, are generally present as small processes from the tube formed by the anterior petals and stamens. Sometimes they are more developed and appear as small lanceolate petals.

Corolla.—C. The Subterranean Cleistogamic Flower. Here the corolla is reduced to a single petal, the posterior ones having quite disappeared, while the anterior alone persists. The hood and its fimbriated process are also lacking. The solitary petal at the time of pollination has the form of a blunt process, about as long as the ovary, viz.,—1 ½ millimeters.

Andrœcium.—A. The Chasmogamic Flower. The stamens are nearly equal, and are placed with the style close under the hood of the anterior petal. The superficial cell-walls of the anthers have stellate thickenings which no doubt assist in dehiscence. From one to two hundred microspores are formed in each anther.

The microspores undergo a great increase in size at the time of flowering. Immediately before the flower opens they are relatively small, with exceedingly thick walls, but they increase several times in size during the maturing of the flower. This seemed a remarkable peculiarity, but numerous measurements of these and exact drawings with the camera lucida, leave no doubt as to the fact. In Plate XVII, Figs. 4–7 is shown a series, taken from successively older blossoms on the same axis, and drawn under the camera. The relative thickness of the wall at first, and the subsequent great increase in size are clearly shown. When ripe, the microspore has increased to several times its bulk and is nearly spherical. It will be seen that the thickened meridional ridges are at first sunken in inrolled grooves, that later they are pressed out to the level of the spore wall and finally come slightly to project. I am not aware that this increase in size has been noted in other plants. An examination of series of pollens from *Hibiscus*, *Rondeletia*, *Clerodendron*, etc., showed that the microspores are nearly constant in size after the bud is well formed.

Andrœcium.—B. The Aërial Cleistogamic Flower. In this the eight stamens typical of the chasmogamic flowers are nearly always represented, part of them in a rudimentary condition. The largest of these cleistogamic anthers, however, are smaller than those of the chasmogamic, and contain a less number of microspores (60–120). The superficial cells show quite clearly the stellate thickenings which aid in dehiscence. The microspores are also smaller, in the ratio of about six to seven. A similar increase in size takes place in them, but the range is not quite so great, and the wall always remains thick.

Andrœcium.—C. The Subterranean Cleistogamic Flower. In this the stamens are greatly reduced both in size and number. Sometimes only two bear perfect microspores. Rudimentary anthers are always found. The number of stamens as well as their development varies, but more than seven seem never to be represented. A well-developed anther may contain 40–80 microspores. The superficial cell-walls of the anther show only a faint indication of the stellate thickenings so prominent on the surface of the same in the chasmogamic flowers. The microspores are still smaller than in the preceding type, as may be seen in Figure 9, and the wall is relatively very thick. This thickness of the microspore wall in the minute flowers seems quite remarkable, and is the reverse of what Darwin and other observers have found in other cleistogamic flowers.

Gynœcium.—A. In the chasmogamic flowers the external appearance is shown in Plate XVII, Fig. 1. The style curiously enough bears two different structures, either of which might be taken for a stigma. Terminally situated is a tuft of hairs amongst which microspores are found, often in a germinating state. Laterally is a glandular knob where the microspores also stick and germinate. Each of these represents the tip of a carpel, but only the latter is functional as a stigma. As the flower opens an abundant secretion is poured forth from the glandular stigmatic knob. The terminal hairy tuft is close to the anthers under the hood. It would seem that the microspores might be caught in this tuft and remain, where they may stick to an insect, leaving the flower after having been smeared with the stigmatic secretion while seeking nectar in the base of the ovary.

A most interesting feature is that in at least many individuals a canal traverses the style, and forms an open passage from the ovarian cavity to the exterior. The stalk of the hairy tuft is a deeply channeled structure, and this channel is continued into a canal in the style proper which below divides into two passages, leading into each cavity of the ovary. These last passages are often nearly choked up with hairy processes from the surrounding tissue. Whether the whole canal can have any function is hard to conjecture, but it suggests the condition seen in the Resedaceæ. Darwin[1] found a similar condition in the cleistogamic flowers of *Viola canina* and *Viola alba.* Of the cleistogamic flower of *Viola canina* he says: "It is remarkable that there is an open passage from the enlarged funnel-shaped extremity to within the ovarium; this was evident, as slight pressure caused a bubble of air which had been drawn in by some accident, to travel freely from one end to the other; a similar passage was observed by Michalet in *Viola alba.*"

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[1] Forms of Flowers, p. 315.

The surface of the ovary bears scattered, club-shaped, glandular hairs, as seen in the figure. Their function is uncertain, but it may be noted in advance that they are much more numerous on the ovaries of the subterranean flower. Stomata are sparingly present on the ovary.

Gynœcium.—B. In the aërial cleistogamic flower the pistil presents a structure which is in a striking manner intermediate between the chasmogamic and subterranean cleistogamic flowers (Plate XVII, Fig. 2). The ovary is smaller than in the former, the style very much shorter, and the hairy tuft greatly reduced, though it still forms a considerable outgrowth. The glandular hairs on the surface of the ovary are rather more abundant than in the preceding type of flower, although, as will be seen later, by no means so profuse as in the underground ones.

Gynœcium.—C. In the subterranean cleistogamic flower the ovary is still smaller than in the last, the knob of the stigma is much reduced in size, the tuft of hairs has almost disappeared, and the style is reduced to the vanishing point (Fig. 3).

The distal surface of the ovary is thickly covered with the peculiar club-shaped glandular hairs already mentioned as being present in the other types of flower. The contrast in this respect with the ovary of the evident flowers is especially striking. The glands are much more numerous, the cavity of each is filled more richly with contents, and together they form almost a complete coating. These glands are occasionally found on the sepals. Nothing can be said positively as to the function of these, but considering their rich development, it must be that here we are dealing with advancing specialization rather than degeneration. Two uses seem possible. They may serve in some way as absorptive organs.

Their rich contents would lend support to this view. Such a suggestion has already been made by Mrs. Pettit[1] in the case of the gynophore hairs of *Arachis hypogæa*. Or since such underground fruits would be especially liable to destruction from animals living in the soil, these glandular bodies may contain some alkaloidal or other substances which may protect the fruit against such. The fact that these bodies are only found on that portion of the ovarian surface which comes into direct contact with the soil as soon as the ovary has grown slightly, might favor the former view. Stomata have never been found on the subterranean ovary.

Since the style scarcely exists in the cleistogamic pistil we should hardly know what to expect in regard to the passage from the ovarian cavity to the exterior. In point of fact openings very frequently occur close beside the stigmatic knob. Until the flowers are quite well developed (nearly ready for pollination), there is always a considerable opening into both chambers of the ovary.

Nectary.—A. In the chasmogamic flower, close under the posterior base of the ovary, there is found growing out from the receptacle a small tract of cells. These are covered by the slightly modified posterior sepal, and receive branches from the vascular system of the peduncle. The surface of the papilla is formed of irregular polygonal cells. Beneath these is a region of cells, elongated perpendicularly to the surface, and lying below them in turn are the terminations of the vascular bundles. There is no doubt as to the character of the structure as a nectary. Insects have been observed to alight on the summit of the corolla and to probe in this region.

Nectary.—B. In the aërial cleistogamic flower the position of the nectary is marked by faintly developed vascular bundles, and in some instances by a small cellular papilla. The development of the latter is however very variable.

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[1] Pettit, *Arachis Hypogæa*. Memoirs of the Torr. Bot. Club, vol. iv, No. 4.

Nectary.—C. In the subterranean cleistogamic flower no certain indication of a nectary has been found.

Seeds.—The evident flowers, as already stated, commonly fail to bring forth seeds. When seeds are produced no difference has been observed in those resulting from the different types of flowers.

The development of the seed-wall presents some points of interest. The individual coats, at the time when they arise as folds growing up over the nucellus, consist over nearly their whole extent of two cell-layers. The inner (secundine) undergoes relatively little development and in the mature state is hard to find. The cells of the outer coat enlarge, and from the first are sharply marked off from the surrounding tissue. At the time of pollination the primine is a two-layered envelope, whose cells are rich in protoplasm and have strongly marked nuclei. Anticlinal cell division takes place rapidly at this time. The cells of the secundine at flowering time are somewhat vacuolated and have small nuclei. From then onward they gradually become larger, vacuolated and degenerate, and may be found only as a thin, imperfect layer lining the hard testa of the ripe seed.

The cells of the primine retain their almost meristematic character, dividing anticlinally, so that although the ovule is swelling rapidly, their axes remain equal. As the corolla withers the two layers composing the primine coat become differentiated: the outer ceases to divide and its cells to enlarge. The inner undergoes still more active anticlinal division than at first so that it becomes a layer of columnar cells perpendicular to the surface. The outer layer begins to develop epidermal processes which in time become the hairy covering. As the ovule approaches its full size the inner columnar cells cease dividing and elongate rapidly till they may fairly be described as needle shaped. Their walls now become greatly indurated, and thus arises the hard coat of the testa. The cells of the outer coat form then a thin zone outside the indurated one, and bear the hairs which give the seed its silvery appearance. It is noteworthy that, long after the indurated layer has become so heavy that it would seem quite impervious to food, the cells of the outer piliferous layer retain their protoplasmic contents and nuclei, and seem like living cells.

Meanwhile the aril has formed as a paired proliferation from the primine close to the funiculus and forms spongy masses of highly vacuolated cells. In the mature seed they are seen as a couple of whitish lobes, rather less than half the length of the seed. The seed is black, 2½–3 millimeters in length, and clothed with hair.

In looking over the structural features it is seen that the aërial cleistogamic flowers show intermediate stages between the other two types alike in form, in position, in development of sepals, petals, stamens and carpels, and in the minuter details of these, the whole constituting perhaps as neat an illustration as is possible, of the stages whereby a plant reaches a highly modified development.

Various experiments have been made by cutting off one or another type of inflorescence to ascertain how far growth correlation and compensation might be exhibited. Plants deprived in early July of their evident racemes, developed others from axillary buds. Removing the subterranean clusters had no visible effect on the plant and they were not replaced. Subterranean flowers that were exposed turned green within three days.

The production of the aërial cleistogamic flowers appears to be dependent on the general vigor of the plant, for in one very dry season they were almost wholly lacking.

POLYGALA PAUCIFOLIA.

As mentioned above, the only other species of the genus *Polygala* which bears cleistogamic flowers is *P. paucifolia*, the so-called Flowering Wintergreen. In appearance and habitat it differs much from *P. polygama.* Described by Willdenow in 1800, references to it in literature have been even more scanty than in the former case.

Polygala paucifolia occurs throughout New England, the Blue Mountains, and the Alleghenies, flowering in Pennsylvania, in the middle and latter part of May. Its vegetative appearance strikingly suggests that of *Gaultheria procumbens,* hence the name Flowering Wintergreen.

From long slender rhizomes which run through the humus, arise at intervals upright shoots 5–8 inches high, and bearing at their summits 3–7 leaves. The rhizomes grow and branch abundantly, so that the plant generally appears in patches. At the tips of the upright shoots are borne the showy flowers, which are much larger than those of *P. polygama,* being in fact by far the largest and most beautiful of our native Polygalas. They average nearly 2 c.m. in length. Only two or three are borne on each vegetative axis, and apparently terminate it. We may now consider the several parts of the flower.

A. Chasmogamic Flower.

Calyx.—This differs from that of most of our native species in being deciduous. This tendency is most marked in the large wings which, properly speaking, are caducous. The three outer remaining sepals are relatively small, greenish white and inconspicuous. The posterior of these is about twice the size of the other two, forming a protective shield for the nectary. Stomata are plentiful on all three. The wings are nearly or quite the length of the corolla (2 cm.), petaloid, light purple, and remain only for a short time widely expanded, falling before the other floral parts. Stomata are present on these also as in the other species, their petaloid character notwithstanding.

Corolla.—The inner whorl of the perianth consists, as before, of one anterior and two posterior petals. The anterior one is hooded, the hood bears a beautiful fringed tuft, and the whole structure measures about 2 cm. in length. The two posterior ones are equal, slightly inferior in length to the fringed hood, and the inner margins of their distal ends are greatly thickened. All three petals fuse below with each other and with the stamens.

Andrœcium.—In this species the stamens are only six in number. They are nearly equal in size; the dehiscence of the microsporangia and the character of the spores are those usual to the genus. The superficial cells of the microsporangial walls exhibit stellate thickenings similar to the condition in *P. polygama.* The microspores are much larger, measuring at the time of pollination about 40 mm. in diameter.

Gynœcium.—Corresponding with the elaborate development of the flower throughout, the pistil is also highly specialized. The two-celled ovary is of the strict *Polygala* type. The style is very long, and just as it leaves the ovary, bends sharply anteriorly, and becomes nearly adherent with the corolla-stamen body. The petals and filaments have united anteriorly in such a way that the style is enclosed in a narrow passage. The tissues of all are more or less united, and so we have here a case of initial gynandry combined with a complete coalescence of petals and stamens. Above, the style expands into a free, relatively large clubshaped organ, which bends posteriorly in a semicircle under the hood and downwards, and bears at its tip a small, glandular, stigmatic point. In this case also a canal traverses the style, and nearly or quite forms a passage from the ovarian cavity to the exterior. The glandular hairs noted on the ovary of the former species are here wanting.

Nectary.—In line with the more showy appearance of *P. paucifolia* we find a highly developed nectary. It occupies the same position as in the former case, and is a receptacular development sheltered by the posterior sepal. To the naked eye it is visible as a prominent bilobed papilla. In section it appears as a many layered cell-mass, receiving branches of the vascular system.

B. CLEISTOGAMIC FLOWER.

At the same time that the conspicuous flowers appear slender, lateral branchlets arise from the leafy shoots near the surface of the soil, bearing a small number of minute flowers. Oddly enough, these branchlets seem as a rule to be apogeotropic, though no exact experiments have yet been attempted.

Only one or two of these lateral branchlets are produced from one axis, and rarely more than three flowers are borne by either of them, so that the number of cleistogamic flowers is very small. The contrast in size between these and the evident flowers is great, the former measuring scarce a millimeter in length.

Calyx.—Five sepals are represented. The lateral ones—wings of the evident flowers—are about twice the size of the other three, which are nearly uniform. All are deciduous. Stomata are present on all, and quite abundantly on the outer surfaces.

Corolla.—There is no trace of any of the petals except the anterior. This is represented by a small process.

Andrœcium.—The six stamens of the chasmogamic flower are commonly all represented, always some of them being in a more or less rudimentary condition. The microspores are also reduced in size, measuring about twenty-five μ in diameter.

Gynœcium.—Here the reduction in size is also relatively very great. The style has almost disappeared, and the ovary is about one-fifth the length of that of the chasmogamic flower. No glandular hairs are present.

Nectary.—No indication of a nectary has been observed in the cleistogamic flowers.

In *P. paucifolia* no intermediate types of flower have as yet been found. It is possible, however, that a more extensive search in its native habitat would reveal the existence of such.

Such studies as these must inevitably raise the inquiry, "Why are cleistogamic flowers developed, and what purpose do they serve in the history of the plant?" Many investigators have studied cleistogamic flowers and several explanations have been offered.

The existence of minute, self-fertilized flowers has been known for several centuries. The first satisfactory description of them was given in 1857 by Dr. Müller, of Upsala,[1] who described the apetalous flowers of *Viola canina.* In 1863 Hugo von Mohl[2] published further observations with the history of the subject. In 1867 Dr. Kuhn[3] gave to such flowers the fitting term "cleistogamic."

We may now review the various opinions which have been held concerning the cause and meaning of these.

Delpino[4] believed that cleistogamic flowers have been developed in order to ensure the production of seeds under climatic or other conditions, which tend to prevent the fertilization of the perfect flowers. Axell[5] supposed similarly that the existence of cleistogamic flowers was due partly to low temperature, which hindered the development of ordinary flowers, and partly to failure of insects which, under normal circumstances, effect cross fertilization. Fritz Müller, in much the same way, believed that cleistogamy was to preserve the existence of the species in case sexual reproduction, depending on favorable outward conditions, failed.

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[1] Botanische Zeitung, 1857, p. 730.

[2] *Ibid.*, 1863, pp. 307–28.

[3] *Ibid.*, 1867, p. 65.

[4] "Sulla Opera la Distribuzione dei Sessi nelle Plante," 1867, p. 30.

[5] "Severin Axell," Om an ordningarna fo̊r fanerogama våxternas befrukting. Stockholm, 1869.

Darwin,[1] quoting Delpino, regards his view to a certain extent as correct. He points out that a majority of plants which produce cleistogamic flowers, have their evident ones adapted for fertilization by insects, and that during certain seasons, through scarcity of insects, these would fail to be fertilized, and so there would be a failure of seed production, unless this were accomplished by some other means. "It is difficult to avoid the belief that the production of cleistogamic flowers, which ensured under all circumstances a full supply of seed, has been in part determined by the perfect flowers being liable to fail of their fertilization." But he immediately makes it clear that he does not consider that this is the chief reason for the development of cleistogamic flowers. He points out that wind-fertilized genera also produce such, and says further of the causes of cleistogamy: "The production of a large supply of seeds with little consumption of nutrient matter and expenditure of vital force, is probably a far more efficient motive power. The whole flower is much reduced in size; but what is more important, an extremely small quantity of pollen has to be formed, as none is lost through the action of insects or the weather, and pollen contains much nitrogen and phosphorus." And seeing that they possess this advantage, he thinks that if any cause prevented a plant from completing the development of its flower, natural selection would step in to preserve this advantage and so lead to the production regularly of cleistogamic flowers.

Mr. S. Le M. Moore[2] criticised Darwin's views on cleistogamy, basing his opinions on a few observations of Vegetable Marrow. He points out that conspicuous flower-parts quickly wither after pollination, and suggests that if pollination for any reason took place before the flower opened, the corolla would cease developing. "I believe that cleistogamy is caused by the physiological condition of great fertility without crossing, coexisting with the morphological one, of germination of the pollen while on the anther cell, or at least before expansion of the perianth. The result of the latter condition is the arrest of the floral envelopes."

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[1] Darwin. Forms of Flowers, p. 339.

[2] Trimen. Jour. of Bot., 1881, p. 84.

Since cleistogamic flowers are often buried in the earth, and so produce fruits which are safe from the danger of being nipped by herbivorous animals, the question of cleistogamy has been identified with that of specially protected forms of fruit. Huth,[1] speaking particularly of the case of *Polygala polygama*, says: "One must look upon this case also as a contrivance for protection against grazing animals." The force of this explanation disappears when one takes into account the aërial cleistogamic flowers. *Polygala polygama* grows largely where tender grasses are not abundant, and suffers much from grazing animals. The green cleistogamic flowers, growing among the foliage, are situated as unfavorably as possible to escape these, and therefore Huth's statement obviously fails to explain them. If any one chooses, he can assume that the two kinds of minute flowers have two different explanations, that the subterranean ones are developed to produce protected seeds, and the aërial ones for another purpose. But if one believes, as does the writer, that one explanation should chiefly cover the whole case in a single plant, the idea of these flowers being developed for the sake of protection must be dropped, except as a secondary condition.

Chodat,[2] also writing of *Polygala polygama* in particular speaks of it as a case of teratology, and differing from other abnormalities only in the frequency of its occurrence. Is it not difficult to suppose, either that this plant is essentially different from others which bear cleistogamic flowers, or that all these are merely teratological cases? Hansgirg[1] dwells on the importance of light and other factors in determining the development of blossoms, and states that flowers may be rendered potentially cleistogamic in several ways. Such a condition he calls pseudocleistogamy and distinguishes varieties according to the character. Kerner[2] regards cleistogamy as due primarily to lack of light. "This result throws some light on the nature of the stimulus which causes the formation of the flowers in question. No open, aërial flowers were produced by *Viola sepincola* so long as it grew in the cool shade of a dense wood, but when transferred to open ground, accessible to sunlight, such flowers were developed. One can hardly err in ascribing to the sun's rays a very important influence in stimulating plants to the inception of flowering shoots, especially such as bear blossoms possessing bright colored petals. Indirectly, however, this advantage accrues to the plants in question that, living as they do in the deep shade, where no bees would, in any case visit them, even if they had open flowers, they can confine their constructive energy to the inception and development of cleistogamous flowers and save themselves the trouble of producing open flowers adapted to cross-pollination, but useless in the place in question. If the spot where the violet grows becomes exposed to the sunlight through the trees shading it being blown down or felled, humble and hive-bees make their appearance in search of honey, and, buzzing from flower to flower, cross the flowers one with another. In such circumstances the open, sweet-scented violet blossoms are in request, and the same plant individual, which for years in the dark shade has developed none but cleistogamic flowers, is now stimulated by the sun's rays into producing flowers with expanded petals.

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[1] *Loc. cit.*

[2] *Loc. cit.*

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[1] Hansgirg. Bot. Central, 45, p. 74.

[2] Natural Hist. of Plants, II, p. 395.

"In the late autumn and early spring, when it is cool, and there are no flower-seeking insects, the Dead-nettle is able to do without the luxury of corollas, which are the means of alluring insects, and as a fact only cleistogamous flowers make their appearance at those seasons. It must not, of course, be imagined that the plant exercises an intelligent discretion of its own when it abandons the development of corollas. The connection between this effect and the aforesaid conditions is indirect, and we must conceive that the nature of the stimulus which results in the inception of flower-buds is different, when a plant is subject to the influence of the short days and the low temperature of late autumn and early spring, from what it is under the conditions prevailing on warm summer days."

Vöchting[1] showed experimentally that the development of flower parts depended much on the presence of light: that if light were diminished the corolla suffered first and later the other floral members. In the case of *Linaria spuria* he has also been able to produce either chasmogamic or cleistogamic flowers at will by regulating light intensity. Schively[2] found that chasmogamic flowers of *Amphicarpæa monoica* never appeared in winter or spring on plants reared in the greenhouse. Only in summer were such produced, and this fact was attributed to the relatively low light intensity of winter. Ludwig[3] expresses the view that cleistogamy is caused by unfavorable weather or lack of insects at flowering time. Engler[4] states that amphicarpy in hermaphrodite flowers is always associated with cleistogamy. In commenting on the fact that where cleistogamic flowers are produced, seed often fails to mature from the chasmogamic ones, he makes the important suggestion in this connection that the developing fruits from the minute flowers require a great quantity of carbohydrates and so hinder the development of fruit from the evident flowers. Knuth[1] reviews the opinions of several of these observers and concludes that light is the important factor in cleistogamy. It will be noticed that Darwin's suggestion that they were developed as a means of economy, does not appear in the modern views.

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[1] Vöchting. Einfluss des Lichtes. Prings. Jahr. xxv, 1893, p. 149.

[2] Schively. *Loc. cit.*

[3] Ludwig. Lehrbuch der Biologie der Pflanzen, p. 427.

[4] Engler. Bot. Central, '95. Beih, p. 265.

Perhaps, therefore, the consensus of opinion at the present time in regard to cleistogamic flowers may be fairly stated as follows: They are caused primarily by deficiency in light, or by other unfavorable conditions, and are to be regarded as degenerate or imperfectly developed chasmogamic flowers. They are of advantage to a plant in preserving the species when ordinary fertilization fails, and in the fact that their fruits are often developed underground where they are protected.

These views either as to causes, or the purpose of cleistogamic flowers are not very satisfactory when applied to such aërial ones as are borne by *Polygala polygama*. In the first place these are produced in abundance in the open fields under the sun of July and August. The plants which bear them show every sign of health, vigor and normal development. If indeed it may have been that lack of light induced their development in the first instance, it is necessary to assume that there is some other cause at work when we find them developed under all circumstances, and under bright sunlight. The view that they are for the purpose of preserving the plant when unfavorable circumstances prevent crossing, is also more or less unsatisfactory, for each summer the cleistogamic flowers are produced in great profusion. One would hardly suppose that the chasmogamic flowers habitually fail to find the conditions to which they are adapted. The fact that the aërial cleistogamic flowers are developed among the green foliage, fairly sets aside also any question of their being a contrivance for protection against herbivorous animals.

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[1] Knuth. Blüthenbiologie.

There is one fact in connection with cleistogamic flowers in general, which it does not seem to the writer has been properly taken into account in theories concerning such. I refer to the rapidity with which the seeds are matured from them. In *Polygala polygama*, much less time is required to produce seed from the cleistogamic flowers, than for the maturing of seed from the evident flowers. The same is true of other plants. In *Amphicarpæa*, for instance, the development of fruit from the cleistogamic flowers is relatively rapid. Some reason must lie back of this phenomenon. It would seem that a cleistogamic flower, requiring a minimum expenditure of material, leaves the shoot prepared to furnish the food very rapidly for the maturing of fruit. Here, as in many other points, Darwin's suggestion after the lapse of years proves the soundest. Cleistogamic flowers are economical in the amount of food required to form the flower parts, and especially in the production of pollen.

Although the observations of Hansgirg, Kerner and Schively leave no room to doubt that light has an influence on the development of the conspicuous parts of blossoms, and even a determining influence on the nature of the flower, as Vöchting demonstrated in *Linaria spuria*, yet these leave the explanation of cleistogamic flowers still incomplete. Some other cause or purpose must exist for the habitual development, under favorable conditions of such numbers of aërial cleistogamic flowers as are put forth by *Polygala polygama* in the sunny weeks of summer.

Considering the rapidity with which the fruits are matured, it seems to the writer that the explanation is chiefly to be found in the relation of cleistogamic flowers to the chasmogamic ones in the matter of food supply.

CONCLUSIONS.

1. *Polygala polygama* develops, in addition to the evident and the subterranean types of flower described in the manuals, a third type—the aërial cleistogamic—which may be found abundantly in midsummer.

The last are morphologically intermediate between the former two types and with occasional transitional forms, furnish a connected series between the conspicuous and subterranean flowers.

The shoots bearing aërial cleistogamic blooms are more or less geotropic.

2. The chasmogamic flowers very largely fail to mature seed. The cleistogamic ones produce seed abundantly.

3. The five *sepals* are present in all types of flower. Only one *petal* (the anterior) is found in the subterranean flowers, and the same, with rudiments of two others (the posterior) appear in the aërial cleistogamic. Eight *stamens* are generally present in the aërial cleistogamic, but more or less reduced, and in the subterranean blooms from three to seven are found, still more reduced, sometimes but two bearing perfect microspores. The *pistil* of the subterranean flowers is greatly reduced; that of the aërial cleistogamic shows a condition intermediate between the former, and that of the chasmogamic flower. A well-developed receptacular *nectary* exists in the chasmogamic flower. Only traces of this are present in the aërial cleistogamic, and it is entirely wanting in the subterranean form.

4. Stomata are present on all parts of the evident calyx, irrespective of color. They are found in extreme abundance on the outer surface of the sepals in the aërial cleistogamic, and are found also on the calyx of the subterranean flowers, but in a rudimentary condition.

5. The microspores of the evident flowers undergo a great increase in size at the time of flowering; the same is true to a less degree of the microspores of the two other types of flower.

6. Contrary to the condition described for the other cleistogamic flowers, the walls of the microspores are very thick.

7. A canal is present in the pistil leading from the ovarian cavity to the exterior. At the base of the style the lumen is sometimes filled by hairy outgrowths from the surrounding tissue. A similar condition is found in *P. paucifolia.*

8. Glandular hairs, found sparsely on the ovary of the evident flowers, are present in great abundance on that of the subterranean ones, pointing to specialization of some kind, possibly a capacity for absorption.

9. The indurated portion of the testa is derived from the inner cell-layer of the primine.

10. The chasmogamic flowers of *P. paucifolia* exhibit a condition of initial gynandry combined with the complete coalescence of stamens and petals.

11. The hypothesis that cleistogamic flowers are developed, to preserve the species when the chasmogamic ones fail, is unsatisfactory, because these are produced each year abundantly.

12. The hypothesis that this development is due to lack of light or similar causes is probably partially true, but is insufficient, because cleistogamic flowers are produced in great abundance on healthy plants in the warm and sunny period of midsummer.

13. Neither can this development be explained as a device for developing protected fruits, for although the plant suffers from grazing animals, these aërial flowers appear among the foliage where the danger is greatest.

14. The cleistogamic flowers, like those of other species, develop seed more rapidly than do the conspicuous ones. It is believed that the purpose of their existence is the economical and speedy production of seed.

EXPLANATION OF PLATES XVI–XVII.

Plate XVI. Plant of *Polygala polygama* as seen in early August. All types of flower are seen. Some of the shoots have been removed.

Plate XVII.

Fig. 1. Pistil from chasmogamic flower.

Fig. 2. Pistil from aërial cleistogamic flower.

Fig. 3. Pistil from subterranean cleistogamic flower. Drawn to scale. On the ovaries are seen the glandular hairs.

Figs. 4a 4b, 5a 5b, 6a 6b, 7a 7b, stages in the enlargement of the microspores of the chasmogamic flower, taken from successive blooms on the same axis. (a), and (b) refer in each case to longitudinal and transverse sections respectively.

Fig. 8a 8b, stage from an aërial cleistogamic flower, corresponding to 6a 6b of the chasmogamic.

Fig. 9a 9b. Corresponding stage in the subterranean cleistogamic. All the microspore sections drawn under camera to same scale.

Fig. 10. Lower epidermis of foliage leaf.

Fig. 11. Lower (outer) epidermis from sepal of chasmogamic flower.

Fig. 12. Lower (outer) epidermis from sepal of aërial cleistogamic flower.

Fig. 13. Lower (outer) epidermis from sepal of subterranean cleistogamic flower. Figs. 10–13 are drawn to scale under camera lucida.

Studies on Growth and Cell Division in the Root of Vicia Faba.

(*WITH PLATE XVIII.*)

BY BLANCHE GARDNER, B. S.

These studies were originally undertaken to determine the growth of the root under varied environmental conditions; but during the progress of the work, several additional interesting lines of study have suggested themselves. So the work has been extended, and can now be grouped under the following heads:

A. Daily Periodicity of Growth in Roots.
B. Relative Growth of Roots in Different Chemical Solutions.
C. Cell Division.

The studies on the root of *Vicia Faba* were suggested to me by Professor J. M. Macfarlane and I wish here to acknowledge my indebtedness to him for suggestions and criticisms of my work.

A. DAILY PERIODICITY OF GROWTH IN ROOTS.

To determine the rate of growth in roots, during day and night, a series of experiments were performed in which seedlings of the broad bean (*Vicia Faba*) and of the pea (*Pisum sativum*) were used. The seeds were germinated in moist sawdust. When the roots reached a length of about one to one and a half inches, they were used for experimentation. Wooden boxes, with one side of glass, were loosely packed with the moist sawdust, and in these the seedlings were placed with the root in a horizontal position against the surface of the glass. Marks could therefore easily and accurately be made on the glass at different hours of the day. The roots were thus arranged, and observations were usually made from 10 A. M. to 4 P. M. and at 9 A. M. the following day. In some experiments, records were taken continuously both during day and night.

The following diagrams (Reference Plates I–III, pp. 180–182) are tracings of the growth of the roots reproduced, from the glass record plates, and the accompanying tables show the environmental conditions of temperature and moisture, which existed at the hours when readings were taken. The rate of growth per hour during day and night is also given.

TABLE I (PLATE I).

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.		No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.					Day.	Night.
1	A. M.	°F.				3	A. M.	°F.			
	10	84	100				9.10	76	100		
	10.20	86	98				10.10	84	100		
	11	86	96				10.40	86	96		
	11.30	87	92				11.10	86	92		
	11.50	88	88				11.30	87	88		
	P. M.						11.50	88	82		
	12.10	89	76				P. M.				
	12.40	89	69				12.10	89	76		
	1	89.5	66				12.40	89	69		
	1.30	90	63				1	89.5	66		
	2	90	60				1.30	90	63		
	2.30	88	59				2	90	60		
	3	86	59				2.30	88	59		
	3.30	86	63				3	86	59		
	4	86	66				3.30	86	63		
	A. M.						4	86	66		
	9	86	96				A. M.				
				$2\frac{1}{6}$ mm.	$\frac{11}{17}$ mm.		9	86	96	2 mm.	$1\frac{3}{7}$ mm.
2	A. M.					4	A. M.				
	9	76	100				9.15	76	100		
	10.10	84	100				10.40	86	96		
	10.40	86	96				11.10	86	92		
	11.10	86	92				11.30	87	88		
	11.30	87	88				11.50	88	82		
	11.50	88	82				P. M.				
	P. M.						12.10	89	76		
	12.10	89	76				12.40	89	69		
	12.40	89	69				1	89.5	66		
	1	89.5	66				1.30	90	63		
	1.30	90	63				2	90	60		
	2	90	60				2.30	88	59		
	2.30	88	59				3	86	59		
	3	86	59				3.30	86	63		
	4	86	66				4	86	66		
	A. M.						A. M.				
	9	86	96				9	86	96		
				$1\frac{3}{4}$ mm.	$\frac{11}{18}$ mm.					2 mm.	$1\frac{7}{10}$ mm.

TABLE I (PLATE I)—*Continued.*

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.
5	A. M.	°F.			
	10.20	86	86		
	10.40	88	80		
	11.15	88	70		
	11.40	88	66		
	P. M.				
	12.15	89	60		
	12.30	89	55		
	1	89	53		
	1.30	88	50		
	2	88	50		
	2.30	86	50		
	3	84	52		
	3.30	84	53		
	4	84	53		
	A. M.				
	9	86	96	2 mm.	$1\frac{1}{17}$ mm.

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.
6	A. M.	°F.			
	10.20	86	86		
	10.40	88	80		
	11.15	88	70		
	11.40	88	66		
	P. M.				
	12.30	89	55		
	1	89	53		
	1.30	88	50		
	2	88	50		
	2.30	86	50		
	3	84	52		
	3.30	84	53		
	4	84	53		
	A. M.				
	9	86	96	$1\frac{3}{11}$ mm.	1 mm.

TABLE II (PLATE II).

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.
7	A. M.				
	10.20	86	86		
	10.40	88	76		
	11.15	88	66		
	11.40	88	60		
	P. M.				
	12.15	89	59		
	12.30	89	55		
	1	89	53		
	1.30	88	50		
	2	88	50		
	2.30	86	52		
	3	84	53		
	3.30	84	53		
	3.50	84	53		
	A. M.				
	9	85	95	$2\frac{4}{11}$ mm.	$1\frac{7}{17}$ mm.
8	A. M.				
	10	72	100		
	P. M.				
	12.10	76	90		
	12.45	76	85		
	1.45	76	79		
	2	80	72		
	2.45	82	67		
	3.15	82	64		
	3.45	80	65		
	4.15	76	67		
	A. M.				
	9	86	95	$1\frac{1}{3}$ mm.	1 mm.

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.
9	A. M.				
	9.30	80	100		
	9.45	82	100		
	10.20	86	100		
	10.45	86	98		
	11.10	88	96		
	11.45	91	95		
	12.30	92	84		
	1.30	88	80		
	2	86	75		
	2.30	84	80		
	3	80	82		
	4	80	82		
	A. M.				
	10	85	100	$1\frac{9}{13}$ mm.	$\frac{5}{8}$ mm.
10	A. M.				
	9.20	66	100		
	10.05	73	100		
	11.10	76	100		
	11.45	80	100		
	P. M.				
	12.20	78	100		
	1	78	100		
	1.40	76	100		
	2	76	99		
	2.30	80	98		
	3	80	98		
	3.30	78	94		
	4	78	95		
	4.30	78	95		
	A. M.				
	9	80	100	$1\frac{13}{14}$ mm.	$\frac{23}{33}$ mm.

TABLE II (PLATE II)—*Continued.*

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.		No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.					Day.	Night.
11	A. M.	°F.				12	A. M.	°F.			
	9.20	66	100				9.30	68	100		
	11.10	76	100				11.10	76	100		
	11.45	80	100				11.45	80	100		
	P. M.						P. M.				
	12.20	78	100				12.20	78	100		
	1	78	100				1	76	100		
	2	76	99				1.40	76	100		
	2.30	80	98				2	76	99		
	3	80	98				2.30	80	98		
	3.30	78	94				3	80	98		
	4	78	95				3.30	78	94		
	4.30	78	95				4	78	95		
	A. M.						4.30	78	95		
	9	80	100				A. M.				
				$1\frac{3}{7}$ mm.	$\frac{7}{11}$ mm.		9	80	100		
										$1\frac{5}{7}$ mm.	$\frac{26}{33}$ mm.

TABLE III (PLATE III).

13	A. M.					15	A. M.				
	9.30	68	100				9.30	68	100		
	11.10	76	100				10.30	78	100		
	11.45	80	100				11.10	76	100		
	P. M.						11.45	76	100		
	12.20	78	100				P. M.				
	1	76	100				12.20	78	100		
	1.40	76	100				1	78	100		
	2	78	100				1.40	76	100		
	2.30	78	100				2	76	100		
	3	78	98				2.30	78	100		
	3.30	78	94				3	86	98		
	4	80	95				3.30	80	94		
	A. M.						4	76	90		
	9	80	96				A. M.		96		
				$1\frac{5}{13}$ mm.	$\frac{12}{17}$ mm.		9	80			
										$1\frac{8}{13}$ mm.	$\frac{11}{17}$ mm.
14	A. M.					16	A. M.				
	9.30	68	100				9.20	66	100		
	10.30	78	100				10.05	68	100		
	11.10	76	100				10.35	73	100		
	11.45	80	100				11.45	80	100		
	P. M.						P. M.				
	12.20	78	100				12.20	78	100		
	1	78	100				1	78	100		
	1.40	76	100				1.40	76	100		
	2	76	100				1.50	76	100		
	2.30	80	100				2	76	100		
	3	86	98				2.30	80	100		
	3.30	80	94				3	80	98		
	4	76	90				3.30	78	98		
	A. M.						4	78	94		
	9	80	96				A. M.				
				$1\frac{3}{7}$ mm.	$\frac{9}{17}$ mm.		9	80	96		
										$1\frac{9}{13}$ mm.	$\frac{7}{17}$ mm.

TABLE III (PLATE III)—*Continued.*

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.		No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.					Day.	Night.
17	A. M.	°F.				18	A. M.	°F.			
	9.20	66	100				9.45	82	100		
	10.05	68	100				10.10	86	100		
	10.30	73	100				11.10	87	90		
	11.45	80	100				11.30	88	80		
	P. M.						P. M.		76		
	12.20	78	100				12	88			
	1	78	100				12.15	88	70		
	1.40	76	100				12.30	88	61		
	2	76	100				12.50	88	64		
	2.30	80	100				1.10	88	64		
	3	80	98				1.50	86	55		
	3.30	78	98				2.30	84	66		
	4	78	94				3	82	70		
	A. M.						4	80	74		
	9	80	96				A. M.				
				$1\frac{6}{13}$ mm.	$\frac{12}{17}$ mm.		9	79	100		
										$1\frac{7}{13}$ mm.	$\frac{6}{17}$ mm.

TABLE IV.

19	A. M.					21	A. M.				
	9.45	82	100				9.30	82	100		
	10.30	86	100				10.30	87	96		
	11.10	86	90				11.10	86	90		
	11.30	86	80				11.30	86	80		
	P. M.						P. M.				
	12	88	76				12.30	88	76		
	12.20	88	68				1.10	88	64		
	1.10	88	64				1.30	87	65		
	1.50	86	65				2	86	65		
	2.10	86	65				2.50	84	67		
	2.30	84	65				3.10	82	70		
	3	82	66				3.40	80	74		
	3.10	80	66				A. M.				
	3.40	80	70				9	79	100		
	4	82	74							$1\frac{3}{4}$ mm.	$1\frac{2}{17}$ mm.
	A. M.										
	9	79	100	$1\frac{7}{12}$ mm.	$\frac{10}{17}$ mm.						
20	A. M.					22	A. M.				
	9.30	82	100				9.50	83	100		
	10.05	83	100				10.10	86	100		
	10.30	87	96				10.30	87	96		
	11.30	86	90				11.10	86	90		
	P. M.						11.30	86	80		
	12	86	88				P. M.				
	12.30	88	76				12	88	76		
	1.10	88	64				12.30	88	68		
	1.50	86	65				1.10	88	67		
	2.30	84	66				1.50	86	65		
	3.10	82	70				2.30	84	66		
	3.40	80	74				3.10	82	70		
	A. M.						3.30	82	74		
	9	79	100				4	80	80		
				$1\frac{7}{12}$ mm.	$1\frac{2}{17}$ mm.		A. M.				
							9	79	100		
										2 mm.	$1\frac{3}{17}$ mm.

TABLE IV—*Continued.*

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.		No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.					Day.	Night.
23	A. M.	°F.				24	A. M.	°F.			
	10.10	86	100				10	84	100		
	10.30	87	96				10.30	87	96		
	11.10	86	90				11.10	86	90		
	11.30	86	80				P. M.				
	11.50	88	80				12	88	76		
	P. M.						12.30	88	66		
	12.10	88	76				1.10	88	67		
	12.30	88	68				1.50	86	65		
	1.10	88	67				2.30	84	65		
	1.50	86	65				3.10	82	66		
	2.30	84	66				3.40	80	70		
	3.10	82	70				A. M.				
	A. M.						9	79	100		
	9	79	100							$1\frac{10}{11}$ mm.	$1\frac{1}{28}$ mm.
				$2\frac{1}{2}$ mm.	$1\frac{1}{18}$ mm.						

TABLE V.

25	A. M.					26	A. M.				
	9.30	68	100				9.30	68	100		
	10.10	70	100				10.45	78	100		
	10.45	78	100				11.45	76	100		
	11.15	78	100				P. M.				
	11.45	79	100				12.15	79	100		
	P. M.						12.45	79	100		
	12.15	79	100				1.15	79	96		
	12.45	79	100				1.45	79	96		
	1.15	79	96				2	79	90		
	2.30	76	90				2.30	76	90		
	3	74	85				3	74	85		
	4	72	82				3.30	72	85		
	6	70	89				4	72	82		
	7	68	90				5	70	85		
	8	68	90				6	70	89		
	9	68	90				7	68	90		
	10	67	90				8	68	90		
	11	66	90				9	68	90		
	12	64	85				10	67	90		
	A. M.						12	66	90		
	3	63	90				A. M.				
	4	64	95				3	64	90		
	5	65	100				4	63	90		
	6			$1\frac{3}{14}$ mm.	$\frac{1}{2}$ mm.		5	64	95		
										$1\frac{3}{14}$ mm.	$\frac{6}{11}$ mm.

TABLE V—*Continued.*

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.		No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.					Day.	Night.
27	A. M.	°F.				29	A. M.	°F.			
	9.15	68	100				9.30	68	100		
	10.10	70	100				10.20	70	100		
	10.45	78	100				11.45	78	100		
	11.15	76	100				P. M.				
	11.45	78	100				12.45	79	100		
	P. M.						1.50	79	96		
	12.15	79	100				2.30	76	90		
	1	79	100				3	74	85		
	1.15	79	96				4	72	82		
	1.30	79	96				6	70	89		
	2	79	90				8	68	90		
	2.30	76	90				9	68	90		
	3.30	72	85				10	68	90		
	5	70	85				11	67	90		
	6	70	89				A. M.				
	7	68	90				1	65	90		
	8	68	90				3	64	85		
	9	68	90				4	63	90		
	11	67	90				5	64	95		
	12	66	90				6	65	100		
	A. M.									$1\frac{1}{15}$ mm.	$\frac{9}{11}$ mm.
	1	65	90								
	3	64	90								
	4	63	90								
	5	64	95								
	6	65	100								
				$1\frac{4}{15}$ mm.	$\frac{21}{22}$ mm.						
	A. M.					30	A. M.				
28	9.30	68	100				9.15	68	100		
	10.20	70	100				10.10	70	100		
	10.40	78	100				10.20	70	100		
	11	76	100				10.45	78	100		
	11.15	78	100				11.15	76	100		
	P. M.						11.45	78	100		
	12.15	79	100				P. M.				
	12.45	79	100				12.15	79	100		
	1.15	79	96				12.45	79	100		
	1.50	79	96				1.30	79	90		
	2.30	76	90				2.30	76	90		
	3	74	85				3.30	72	85		
	3.30	72	85				5	70	85		
	4.15	72	82				7	68	89		
	5	70	85				8	68	90		
	6	70	89				9	68	90		
	7	68	90				11	67	90		
	8	68	90				12	66	90		
	9	68	90				A. M.				
	10	67	90				3	65	85		
	11	67	90				4	64	90		
	A. M.						5	64	95		
	1	65	90				6	65	100		
	3	64	85							1 mm.	½ mm.
	4	63	90								
	5	64	95								
	6	65	100								
				$1\frac{2}{3}$ mm.	$\frac{9}{11}$ mm.						

TABLE VI.

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.		No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.					Day.	Night.
31	A. M.	°F.				33	A. M.				
	9,50	68	100				9.45	68	100		
	10.45	78	100				11.15	76	100		
	11.45	78	100				11.45	78	100		
	P. M.						P. M.				
	12.45	79	100				12.15	78	100		
	1.15	79	100				12.45	79	100		
	1.30	79	100				1.15	79	100		
	2.30	76	90				1.30	79	100		
	3.30	72	85				2	79	90		
	4	72	82				2.30	76	98		
	4.50	70	85				3	74	85		
	6	70	89				3.30	72	85		
	10	68	90				4.15	72	82		
	12	66	90				5	70	85		
	A. M.						7	68	90		
	3	64	90				8	68	90		
	4	63	90				9	68	90		
	5	64	95				10	68	90		
				1$\frac{6}{7}$ mm.	$\frac{7}{11}$ mm.		11	67	90		
							12	66	90		
							A. M.				
							1	65	85		
							5	63	90		
							6	65	95		
										1$\frac{3}{7}$ mm.	$\frac{8}{11}$ mm.
32	A. M.					34	A. M.	°F.			
	9.45	68	100				10	70	100		
	11.45	78	100				10.20	70	100		
	P. M.						11.15	76	100		
	12.45	79	100				11.45	78	100		
	1.50	79	100				P. M.				
	3	74	90				12.45	79	100		
	3.30	72	85				1.15	79	100		
	4	72	82				2.30	76	90		
	5	70	85				3	74	85		
	7	68	90				3.30	72	85		
	8	68	90				4.15	72	82		
	9	68	90				6	70	89		
	10.00	68	90				7	68	90		
	10.45	68	90				8	68	90		
	11	67	90				9	68	90		
	11.45	67	90				10	68	90		
	12	66	90				11	67	90		
	A. M.						12	66	90		
	3	64	85				A. M.				
	5	63	90				1	65	90		
	6	64	95				2	65	90		
				1 mm.	$\frac{6}{11}$ mm.		3	64	85		
							4	63	90		
							5	64	90		
							6	65	95		
										1$\frac{7}{12}$ mm.	$\frac{7}{8}$ mm.

TABLE VI—*Continued.*

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.
35	A. M.	°F.			
	9.45	68	100		
	11.15	76	100		
	11.45	78	100		
	P. M.				
	12.45	79	100		
	1.15	79	100		
	2	79	100		
	2.30	76	90		
	3	74	85		
	4	72	85		
	5	70	85		
	6	68	89		
	7	68	90		
	8	68	90		
	9	68	90		
	10	68	90		
	11	67	90		
	12	66	90		
	A. M.				
	1	65	90		
	4	63	90		
	6	65	95		
				2 mm.	$\frac{8}{13}$ mm.

No. of diagram.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.
36	A. M.	°F.			
	9.30	68	100		
	10.25	70	100		
	10.40	76	100		
	11.15	78	100		
	11 45	79	100		
	P. M.				
	12.15	79	100		
	12.45	79	100		
	1.15	79	100		
	1.50	79	100		
	2.30	76	90		
	3.	74	85		
	3.30	72	85		
	4.	72	82		
	5.	70	85		
	6.	68	89		
	7.	68	90		
	8.	68	90		
	9.	68	90		
	10.	68	90		
	11.	67	90		
	12.	66	90		
	A. M.				
	1.	65	90		
	4.	63	85		
	5.	64	90		
	6.	65	95		
				2¾ mm.	1$\frac{1}{12}$ mm.

To prove that the placing of the root in the horizontal position did not in any way cause a peculiar result, experiments were performed in which the roots were from the beginning allowed to grow in a vertical direction. These were performed under similar conditions. The results were the same as those in which the roots were placed horizontally. The following tables show the results obtained :

TABLE VII.

No. of experiment.	Time.	Temperature.	Moisture.	Rate of growth per hour.		No. of experiment.	Time.	Temperature.	Moisture.	Rate of growth per hour.	
				Day.	Night.					Day.	Night.
1	A. M.	°F.				4	A. M.	°F.			
	9	88					9	88			
	10	91					11	95			
	11	95					P. M.				
	P. M.						12	98			
	12	98					1	99			
	1	99					3	95			
	2	96					4	93			
	4	93					A. M.				
	A. M.						9	86		$\frac{5}{7}$ mm.	$\frac{7}{17}$ mm.
	9	86		$1\frac{1}{7}$ mm.	$\frac{6}{17}$ mm.						
2	A. M.					5	A. M.				
	9	88					9	88			
	10	91					10	91			
	11	95					11	95			
	P. M.						P. M.				
	12	98					12	98			
	1	99					1	99			
	2	96					2	96			
	4	93					4	93			
	A. M.						A. M.				
	9	86		$\frac{13}{14}$ mm.	$\frac{6}{17}$ mm.		9	86		1 mm.	$\frac{7}{17}$ mm.
3	A. M.					6	A. M.				
	9	88					9	88			
	10	91					11	95			
	11	95					P. M.				
	P. M.						12	98			
	12	98					1	99			
	1	99					3	95			
	2	96					A. M.				
	4	93					9	86			
	A. M.										
	9	86		$1\frac{1}{7}$ mm.	$\frac{9}{17}$ mm.					$\frac{5}{6}$ mm.	$\frac{1}{3}$ mm.

The conclusion to be drawn from the accompanying figures is very evident; *i. e.*, that without exception the growth of the roots is greater during day than during night. In all the diagrams there is seen a change in position of the root, indicated by the dotted line. This is due in all probability to the circumnutation of the roots, and in calculating the rate of growth, all such changes have been taken into consideration.

Many more experiments were performed than are here shown, and in *every* case the result was the same. Besides these experiments with the bean, more than fifty others were performed with the pea, and records of these were similarly made. The results here confirm those of the bean. In many cases they are even more striking. The following table gives the rate of growth per hour during day and night of twenty peas:

Rate of growth per hour.		Rate of growth per hour.	
Day.	Night.	Day.	Night.
$2\frac{1}{6}$ mm.	$1\frac{1}{17}$ mm.	$1\frac{10}{11}$ mm.	-12 mm.
2 "	1 "		$\overline{+7}$ "
2 "	$1\frac{2}{17}$ "	$2\frac{4}{11}$ "	$-\frac{33}{34}$ "
$2\frac{4}{17}$ "	$\frac{9}{17}$ "	$3\frac{3}{11}$ "	$1\frac{15}{34}$ "
$1\frac{1}{3}$ "	$\frac{12}{17}$ "	$1\frac{1}{3}$ "	$\frac{12}{17}$ "
$1\frac{11}{27}$ "	$\frac{11}{17}$ "	$1\frac{3}{4}$ "	$\frac{1}{2}$ "
$2\frac{2}{27}$ "	$\frac{10}{17}$ "	$1\frac{1}{3}$ "	$\frac{16}{17}$ "
$1\frac{25}{27}$ "	$\frac{31}{34}$ "	$1\frac{1}{2}$ "	$\frac{15}{17}$ "
2 "	$\frac{13}{17}$ "	2 "	$\frac{14}{17}$ "
$2\frac{2}{13}$ "	$\frac{33}{34}$ "	2 "	$\frac{9}{17}$ "
		2 "	$\frac{11}{17}$ "

The results, which the writer obtained, are in direct opposition to those obtained by Sachs. Sachs believed that growth was greater during night than during day. He says that although the increased temperature of the day would induce an increase in the growth by day, yet daylight works in the opposite way. In all his experiments and diagrams, he shows an increase of growth during night and a progressive diminution during day.

Another writer, Walter Maxwell, in a paper entitled "The Rate and Mode of Growth of the Banana Leaf,"[1] has worked on the same question. His experiments, though conducted on a different organ of the plant, agree fundamentally with those given in the present paper. He says: "In comparing the growth of leaves by day and night 70 per cent of the total growth takes place between 7.30 a. m. and 5.30 p. m.," or, in short, growth by day exceeds that by night.

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[1] Bot. Cent. 1896, Vol. 67, p. 1.

It seems difficult to account for such results as those obtained by Sachs. The experiments, performed by the writer, were conducted under perfectly natural environmental conditions, and moreover, not one of the results would stand as an exception to the general conclusion. And as proof that no extraneous or mechanical factors could have interfered during the night, are the experiments watched continually during the entire period of twenty-four hours. The latter are in no way different from those in which observations were made during the day until 4 or 5 p. m., and where the night growth was obtained by the reading of the next morning.

The rate of growth was obtained by measuring the amount of growth during a definite number of hours of the day or night, and calculating from this the rate per hour. Thus, for example, to take the root, recorded on Plate I, Fig. 1—here the growth from 10 a. m. to 4 p. m., or for six hours, was 13 mm.,—a rate of $2\frac{1}{6}$ mm. per hour, and from 4 p. m. to 9 a. m. of the following day,—or for a period of 17 hours,—the growth was 11 mm.,—a rate of $\frac{11}{17}$ mm. per hour.

From the above experiments and observations, but one conclusion can be drawn, and this, as before mentioned, is the fact that the rate of growth of roots is greater during day than during night.

B. RELATIVE GROWTH OF ROOTS IN DIFFERENT CHEMICAL SOLUTIONS.

These experiments were performed with a view to ascertain the action of chemical solutions of various strengths on the growth of germinating seedlings.

Dr. Rodney True[1] has recently published some instructive results on the toxic action of acids and salts on seedlings.

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[1] Toxic Action of Dissolved Salts. R. True. Bot. Gazette, vol. 22, 1896.

His work, however, is not precisely in the same line as that of the writer, for his experiments were performed to show that the toxic action of solutions of electrolytes is due to the action of the ions present.

Mr. T. D. Heald, in a paper entitled "Toxic Effect of Acids and Salts upon Plants,"[2] has shown the effect of various chemical solutions on seedlings. He finds that the relative sensitivity to acid poisons is as follows:

Pisum sativum seedlings were killed in solution $\frac{N}{6400}$
Zea Mays " " " " " $\frac{N}{1600}$
Cucurbita Pepo " " " " " $\frac{N}{3200}$

When expressed in the form of per cent the extremely small amount of acid necessary to kill *Pisum sativum* seedlings is even more striking, and may be expressed as follows:

HCl	0.00056 per cent.
H_2SO_4	0.00076 per cent.
HNO_3	0.00098 per cent.
HBr	0.00126 per cent.

He further experimented with a long series of salts of Nickel, Cobalt, Copper, Mercury, Gold, etc., and showed the toxic action to be due to the ions into which the substances split up, in great dilution.

The writer's experiments were performed with solutions of HCl, $NaCl$, $(NH_4)_2CO_3$, of strengths varying from two to one one-hundredth per cent. Seedlings of the pea, bean and corn were used. These were placed in pots filled with thoroughly washed asbestos, and the asbestos was kept moist with the respective solutions. Asbestos was used in preference to sawdust to prevent any fermentation or chemical reaction, which the latter when in contact with the solutions might induce.

The growth of the root, *i. e.*, the increase in length, was measured daily. Every set of experiments includes a control in which ordinary water is used, so that with this as a standard, the increased or retarded growth caused by the use of the chemical solutions, can be readily perceived. The following tables show the daily increase in the length of the roots in the various solutions :

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[2] Bot. Gazette, vol. 22, 1896.

Feb. 7.	9	10	12	13	14	15	Dec. 13.	15	16	18	19
	mm.							mm.			
H_2O.	7	20	38	40	56	75	H_2O.	3	11	41	60
	6	13	30	37	50	60		6	16	32	46
Peas.	2	11	35	50	60	80	Peas.	6	23	45	58
	4	13	18	32	36	60		9	16	35	54
	...	3	26	37	45	56		...	...	20	32
Beans.	...	1	25	33	42	59	Beans.	...	...	15	30
	...	...	30	44	56	77					
NaCl ½%.	7	18	35	42	50	65	NaCl ½%.	9	20	55	61
	6	19	40	52	62	76		3	12	30	45
Peas.	6	19	32	36	45	56	Peas.	9	19	47	56
	6	14	23	30	40	55		2	12	30	40
	...	...	40	50	55	60		...	...	22	36
Beans.	...	...	13	26	24	35	Beans.	...	...	13	26
	...	2	30	58	82	101		...	...	29	34
NaCl ⅓%.	7	18	59	72	87	106	NaCl ⅓%.	6	19	47	50
	7	20	78	82	95	110		2	11	46	57
Peas.	6	14	35	43	60	79	Peas.	1	12	46	53
	6	18	40	52	65	85		6	20	38	58
	...	...	5	34	45	56		...	1	17	40
Beans.	...	3	46	52	56	67	Beans.	...	...	20	51
	...	...	24	40	47	64		...	...	12	31
NaCl ⅕%.	4	11	65	76	94	104	NaCl ⅕%.	4	21	70	97
	7	17	55	70	86	100		7	19	65	75
Peas.	6	15	42	43	50	59	Peas.	14	25	55	73
	7	19	35	37	74	92		7	15	40	74
	...	...	25	35	45	60		...	2	25	50
Beans.	...	...	15	22	30	53	Beans.	...	1	23	47
	...	...	18	32	47	63		...	1	25	51
NaCl $\frac{1}{10}$%.	7	15	60	72	91	106					
	7	20	53	65	80	93					
Peas.	6	14	40	62	70	87					
	7	17	40	47	65	77					
	...	...	36	46	72	77					
Beans.	...	3	44	58	71	86					
	...	...	75	83	100	106					
NaCl $\frac{1}{20}$%.	7	31	56	76	83	96					
	15	29	60	78	97	120					
Peas.	10	27	40	67	80	92					
	6	13	37	50	67	87					
	...	1	30	47	55	87					
Beans.	...	1	40	50	63	77					
	...	3	45	58	74	100					

Feb. 7.	9	10	12	13	14	15
H_2O.	2	18	28	31	48	75
	7	13	32	40	52	65
Peas.	5	12	30	35	45	54
	1	14	20	35	47	51
	. . .	. . .	20	37	58	72
Beans.	. . .	. . .	13	20	30	53
	. . .	. . .	20	37	54	62
HCl $\frac{1}{2}$%.	5	18	37	50	61	67
	4	16	25	39	67	81
Peas.	5	15	30	40	57	66
	4	14	33	45	63	78
	. . .	. . .	23	33	46	57
Beans.	. . .	. . .	37	44	60	70
	. . .	. . .	10	26	45	55
HCl $\frac{1}{4}$%.	1	12	44	55	66	87
	13	25	37	50	65	86
Peas.	7	15	35	46	55	70
	. . .	4	50	73	95	100
Beans.	. . .	0	54	61	77	90
	. . .	8	60	71	87	100
HCl $\frac{1}{8}$%.	2	14	42	55	75	95
	6	13	43	65	85	100
Peas.	6	15	40	65	89	96
	7	15	32	53	78	91
	. . .	. . .	50	72	101	120
Beans.	. . .	. . .	47	57	77	92
	. . .	. . .	50	64	85	93
HCl $\frac{1}{10}$%.	10	22	55	62	86	110
	17	24	65	85	107	130
Peas.	6	28	36	57	78	87
	8	17	30	46	67	92
	. . .	4	46	66	86	110
Beans.	. . .	3	35	60	77	90
	. . .	. . .	28	40	58	79
HCl $\frac{1}{20}$%.	7	19	52	71	96	125
	5	15	27	55	82	104
Peas.	6	16	57	75	97	135
	. . .	. . .	52	70	96	112
Beans.	. . .	. . .	60	80	90	110
	. . .	. . .	27	55	78	92

Dec. 13.	15	16	18	19
H_2O.	3	11	41	60
	6	16	32	46
Peas.	6	23	45	58
	9	16	35	54
	. . .	. . .	20	32
Beans.	. . .	. . .	15	30
	. . .	. . .	. . .	. . .
HCl $\frac{1}{2}$%.	1	12	45	66
	1	13	40	47
Peas.	4	16	50	65
	2	14	55	60
	. . .	. . .	20	40
Beans.	. . .	. . .	31	42
	. . .	. . .	17	30
HCl $\frac{1}{4}$%.	18	31	76	96
	11	22	65	86
Peas.	13	16	59	74
	3	15	57	70
	. . .	. . .	36	50
Beans.	. . .	. . .	30	47
HCl $\frac{1}{8}$%.	2	20	45	70
	11	32	56	96
Peas.	9	30	50	87
	. . .	. . .	40	50
Beans.	. . .	. . .	35	60

March.	3	5	6	7	8	March.	22	23	25	26	27	28
H_2O.	7	16	19	35	55	H_2O	2	13	41	60	85	99
	6	16	25	36	52		3	16	35	70	82	97
Peas.	0	10	42	52	69	Corn.	4	16	45	65	85	100
	6	12	17	30	40		2	13	37	43	60	86
	. .	11	36	40	55		. . .	10	35	40	48	60
Beans.	. .	25	37	46	69	Beans.	. . .	20	30	38	40	55
	. .	30	15	78	87		. . .	19	35	42	57	69
$(NH_4)_2CO_3$	1	12	16	25	30	$(NH_4)_2CO_3$	4	6	12	15	20	30
$\frac{1}{30}$%.	1	11	15	23	32	$\frac{1}{30}$%.	1	4	10	21	29	35
Peas.	10	20	27	30	40	Corn.	1	5	15	25	30	34
	5	15	25	29	28		2	5	10	20	28	39
	. .	23	31	42	46		. . .	1	22	30	43	49
Beans.	. .	19	34	47	60	Beans.	. . .	1	8	26	30	40
	. .	3	18	28	40		. . .	3	18	28	38	48
$(NH_4)_2CO_3$	12	14	20	40	50	$(NH_4)_2CO_3$	3	26	36	50	75	85
$\frac{1}{50}$%.	1	11	36	52	70	$\frac{1}{50}$%.	1	17	40	52	75	96
Peas.	6	17	36	50	71	Corn.	3	13	53	70	82	95
	2	13	23	41	49		4	20	50	65	79	97
	. .	30	37	46	69		. . .	25	35	50	68	80
Beans.	. .	36	40	60	86	Beans.	. . .	8	10	21	36	59
	. .	11	36	40	56		. . .	12	30	31	50	70
$(NH_4)_2CO_3$	12	20	26	40	65	$(NH_4)_2CO_3$	2	23	65	105	130	160
$\frac{1}{100}$%.	10	21	37	49	78	$\frac{1}{100}$%	13	27	60	85	108	140
Peas.	13	30	47	65	82	Corn.	9	28	61	82	96	120
	11	20	40	63	89		10	28	70	98	125	153
	. .	40	66	95	125		. . .	5	20	33	55	76
Beans.	. .	24	35	45	75	Beans.	. . .	3	20	36	59	80
	. .	38	43	59	95		. . .	2	28	37	60	85

The conclusions have been derived from many more experiments than are indicated by the above tables. Yet those given represent the typical and normal results and also show the mode of experimentation.

NaCl.—Peas and beans will germinate in NaCl solutions of strengths as high as two per cent. In stronger solutions germination rarely occurs. A solution of two per cent or one per cent has a toxic influence and causes the seedlings soon to shrivel. A one-half per cent to a one-third per cent solution can be regarded as neutral, *i. e.*, a solution which neither retards nor accelerates growth, or one which acts like ordinary atmospheric moisture. All solutions weaker than one-third per cent act as stimulants to the seedling and cause an increased growth. From the tables can readily be seen the increase in growth of the roots, in solutions of one-fifth, one-tenth and one-twentieth per cent above the control.

HCl.—Seedlings will germinate, but will live, for only a comparatively short time in solutions of HCl of one or two per cent. The neutral point is reached at a one-half per cent solution. All weaker solutions such as one-fourth per cent, one-fifth per cent, one-tenth per cent act as stimulants.

$(NH_4)_2CO_3$.—In using $(NH_4)_2CO_3$ it was found that only when very weak solutions were used, was it possible to obtain any results. Germination never occurred in solution of one-half per cent, or one-fourth or even one-tenth per cent. Seeds germinate and live in a one-thirtieth per cent solution, yet this growth is a retarded one. The neutral point is reached in solution of about one-fiftieth per cent and all weaker solutions act as stimulants. One seventy-fifth per cent and one one-hundreth per cent were used, both causing a marked increase in growth.

So we must conclude that it is possible to obtain chemical solutions of such a strength that they act as nutritive stimulants, causing an acceleration in the growth—also to find the points of these same solutions which can be regarded as neutral, and above which point these solutions have a toxic or retarding action.

C. Cell Division.

Within the past thirty years cell division has been a favorite study for cytologists. There has always been much difference of opinion amongst writers as to what is the correct explanation of their observations. Before stating my own observations and conclusions, a short review of the more recent papers will be given from which can be seen how greatly authors differ on the subject.

A valuable cytological paper which reviews and condenses the general literature on cell division, is that of Dr. T. Montgomery, entitled, "Comparative Cytological Studies with Especial Regard to the Morphology of the Nucleolus." His observations are based upon animal cells and his conclusions, briefly stated, are as follows :

Number of Nucleoli.—The nucleoli vary in number from one to five and in certain stages of some cells—there may be several hundred. In a few cells no nucleoli are present.

Position of the Nucleolus in the Nucleus.—When a single nucleolus is present it is always placed excentrically though not against the nuclear membrane. Those cases where it occupies the centre of the nucleus are to be regarded as exceptional. In regard to this point the author says he is unable to agree with Professor Macfarlane, who believes the nucleolus to be the morphological and trophic center of the cell.

The nucleolus is often suspended in the chromatin network, but not in such a way that the fibers penetrate its substance ; they are merely wound around it.

General Morphological Structure of the Nucleolus.—The nucleolus may or may not have a membrane. Vacuoles are normal structures in nucleoli since they may be seen after the most diverse methods of fixation. To the nucleolini or endonucleoli which Professor Macfarlane regards as "the tropic center of the cell and as an important mechanical agent during nuclear division," the author attaches no morphological significance.

Amœboid movements have been frequently observed in nucleoli and these are in all probability normal, and perhaps should be considered as an inherent function of the nucleolus, since no movements in other parts of the nucleus are known in a resting cell.

Nucleolar division is of two kinds : (1) The mode by which the nucleolus becomes elongated and then breaks into two or more parts whereby the daughter nucleoli are usually capable of further division, and (2) the mode in which the nucleolus fragments nearly simultaneously into a number of small granules. The author regards the second mode as a process of degeneration.

Fusion of Nucleoli.—This is not as widely known as division, yet it is not unusual. The nucleolus at the time of its origin may be said to be undergoing a process of fusion, since it is produced by the coalescence of numerous small portions of nucleolar substance.

Origin of Nucleolus.—In ova of Nemerteans, nucleoli always appear in contact with the nuclear membrane, then wander toward the center. This appearance of the nucleolus near the nuclear membrane is explained by the fact that the nucleolus is extranuclear in origin. The author says he has found no evidence that the nucleoli derive any part of their substance from the chromatin. The nucleus seems to assimilate some substance from the cytoplasm, and after this has entered the nucleus it undergoes a chemical change, and is deposited on the inner surface of the nuclear membrane in the form of masses of varying dimensions. Its origin is extranuclear, and though it may undergo chemical changes after entering the nucleus, it can be regarded neither as an excretion nor a secretion of the latter.

Behavior of the Nucleolus during Nuclear Division.—During mitosis, the nucleolus may either not disappear or it may disappear before the spindle has formed. In cases where it does not disappear, it divides in two, each daughter nucleus receives half. The usual mode of behavior is for the nucleolus to disappear. It either diminishes in size and then vanishes, or it first fragments into a number of smaller pieces which then disappear. The dissolution of the nucleolar substance commences before the nuclear membrane has disappeared and by the time it has disappeared all the nucleolar substance is dissolved by the action of the cytoplasm or dispersed through the latter, so that no remnant is found in the region of the spindle or of the chromosomes. During the time the nucleolar substance is disappearing, the chromatin stains red with eosin and this the author says may be explained by the assumption that the nucleolar substance either unites chemically with the chromatin or penetrates into the meshes of the latter. There is, however, no chemical union with the chromatin and, therefore, the chromosomes probably do not serve to carry it over to the daughter nuclei.

There are only a few observations which would show that the chromosomes are derived from nucleoli. But most observers agree that the nucleoli disappear more or less during mitosis, and that the chromosomes are not derived from them. As to the mode of transference of nucleolar substance to the daughter nuclei, the author concludes that it is different in different objects. In most cases, the nucleolus disappears. Wager suggests that the chromosomes serve for mechanical vehicles of transportation. Or the nucleolus may become dispersed in the cytoplasm after the nuclear membrane has disappeared and that each daughter nucleus may take up substance from the cytoplasm or produce its own nucleolus from new substance.

Function of the Nucleolus.—As a relatively large amount of nucleolar substance is found in the growing nuclei, the author concludes that it stands in some connection with the process of nutrition, is either itself nutritive or represents that portion of the nucleus from which all the nutritive material has been extracted, *i. e.*, it is a waste product. Or it may represent accumulations of nutritive material retained in the nucleus as a reserve supply—this last is not very probable.

Dr. Montgomery suggests the hypothesis that the nucleolus may arise as a functionless inert mass and acquire its activity later. But in all cases the nucleolus would seem to be in direct connection with the nutritive substance and forces of the nucleus.

Next Strasburger's views will be given as taken from his paper "Ueber Cytoplasmastructuren, Kern und Zelltheilung."

In pollen mother cells of *Larix*, in preparations stained with saffranin and gentian orange, Strasburger observed that at first the kinoplasm was stained violet, but that this violet color disappeared simultaneously with the aggregation of the nucleolar substance in the nucleus. This suggested that the nucleolar substance stood in relation with the activity of the kinoplasm. These observations were confirmed by the internal relations which the nuclei in *Fucus*, *Sphaeroplea*, and the asci of the Ascomycetes showed to the formation of the aster. Between the nucleus and kinoplasm exists, therefore, a close relationship, and on these observations he founds the view that the nucleolar substance represents a reserve material, from which the kinoplasm derives its activity. R. Hertwig's observations show the relations which exist between nucleolar substance and the chromosomes—for he saw that the nucleolus disappeared as the chromosomes became distinct.

Professor J. M. Macfarlane, in a paper, "The Structure of Plant Hybrids and Parents," gives the following, which is a concise statement of his views, clearly stating the position taken by him:

"The nucleolus is the special chromatic and cell center. It forms the main mass of chromatin substance, and is connected by an extremely fine network system with the nuclear membrane which is also chromatic, and during division breaks down to fuse with the radiating threads from the nucleolus. In the reformation of the daughter nuclei also, round the nucleoli the nuclear membrane reappears first on the outer pole or nuclear face, but some can be traced to the pyrenoid centers."

We may now give a short review of an article by H. H. Dixon, entitled, "The Possible Function of the Nucleolus in Heredity."[1] His conclusions have been derived not only from his own work, but from a careful consideration of the views of numerous writers.

The majority of biologists believe that the hereditary substance is transmitted in the chromatin of the nuclear thread, this suggestion coming first from Strasburger. The above writer extends this generally accepted theory. According to him, the hereditary substance is contained completely in the chromatin elements (chromosomes) during nuclear division, but during the resting stage of the nucleus it is suggested as probable that the hereditary substance is distributed between the chromatin thread and the nucleoli. The hereditary substances (idioblasts), which determine the attributes of the cell in which they are situated, are located in the chromatin thread, while inactive idioblasts are in the nucleolus or nucleoli.

Hertwig says that to prove the above hypothesis four points must be considered:

1. The equivalance of the male and female hereditary masses.
2. The equal distribution of the multiplying hereditary masses upon the cells, derived from the fertilized ovum.
3. Prevention of the summation of the hereditary masses.
4. The isotropism of the protoplasm.

The opinion that the substance of the nucleolus is distributed along the chromosomes, during the early stages of karyokinesis, has been gaining favor. The evidence in favor of this view is based on the simultaneous change in the amount of nucleic acid in the chromatin and the nucleoli. As the latter decreases in amount, the former increases, and *vice versa.*

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[1] Annals of Botany, June, 1898.

According to this, Hertwig's first condition is assured, for if chromatin alone were regarded as the bearer of hereditary substances the equivalence cannot be retained.

The second condition is likewise assured. The hereditary mass being composed of nucleoli and chromatin, is equally distributed, during karyokinesis, among the cells derived from the fertilized ovum.

Those who consider the chromatin to be the sole hereditary substance believe that the elimination of hereditary substance, which would be necessary to prevent a summation of the hereditary masses, is effected by the so-called "division with reduction." But this latter theory is not yet established on a sure basis.

The fourth condition affects Strasburger's nuclear hypothesis and the proposed extension of it equally.

Therefore, judged from Hertwig's criterion, this theory regarding the chromatin and nucleolus, as hereditary in function, finds no objections.

Mature tissues contain relatively a large amount of nucleolar substance and a relatively small amount of chromatin. As the cell becomes specialized, the chromatin is reduced in quantity until the idioblasts representing the special properties of the mature cell are alone present.

The great surface of the chromatin as compared with the small surface of the nucleolar substance favors the view that chromatin may be composed of active idioblasts. The greatly attenuated thread, described by Rosen, is explained readily by this theory. This long chromatin thread is immersed in a nuclear fluid containing a large amount of nucleolar substance suspended in it, and thus a uniform distribution of idioblasts along this thread is possible. The persisting portion of the nucleolus contains that portion of hereditary substance which is to be got rid of and not to appear in succeeding generations.

The transformation of the nucleoli, bodily into nuclei, in the spores of *Saccharomyces*, as observed by Wager, is a strong argument in favor of the view here put forward. The author's own observations on the ascospores of *Tuber æstivum* confirm this. He has observed, that at first the nuclei formed in the ascus are large, and contain a network of chromatin, in which a few nucleoli stained with acid anilin dyes, are imbedded. The nucleus enlarges, the chromatin disappears, whilst the nucleoli increase in size and number, and now exhibit an affinity for basic stains. In this process, there is probably at first a transference of chromatin into nucleoli and afterward a bodily transference of these latter into nuclei.

In all these cases, if we admit the hereditary functions of the chromatin, it is scarcely possible to deny it to the nucleoli.

My own observations were made on the cells in the growing apex of the root of the broad bean (*Vicia Faba*).

METHODS.

As fixatives chromic and chrome-acetic acids were employed, both of which gave excellent results. These solutions are washed out with water till all the yellow color has disappeared. The material is then gradually passed through alcohols of increasing strength into pure alcohol. As a clearing fluid, oil of cedar was used, as it is less liable to cause shrinkage, than does xylol. First the material was put into a mixture of the oil and pure alcohol, next into pure oil, thereafter into paraffin, several changes in this last being made to insure complete elimination of the oil. All changes from one solution to another must be made gradually, as for example, from the alcohol to the oil. Sections were cut on the microtome with a feed of six μ. All staining was done on the slide; the best stain employed, and the one from which all sketches have been made was iron hæmatoxylin and Bordeaux red.

OBSERVATIONS.

The Resting Cells.—These are large and enclose a well-formed nucleus. The nuclear substance, or nucleoplasm is stained a bluish crimson. The nuclear substance of the cells in the bean root contains but a very small quantity of chromatin. By careful focusing it is possible to distinguish minute chromatin granules, stained somewhat darker than the very faint linin threads. The nucleolus is a large, rounded mass, and stains a dense bluish black. It may be in the centre of the nucleus, or as is more usual, it is excentrically placed.

Number of Nucleoli.—There may be one nucleolus present, or more than one. The usual number in cells in the growing apex is one, in cells back of the apex, it is as common a feature to have two, as it is to have one. Very rarely are three large nucleoli to be found. Frequently, in nuclei with one or two large nucleoli, many small nucleolar fragments are found. These smaller pieces are always recognizable as nucleolar material by their density and staining relations.

DIVISION.

The *first traces* of division are to be seen in an aggregation of the nuclear material (Plate XVIII, Fig 6.). This aggregation always occurs previous to the formation of the spirem thread. The minute chromatin granules becoming more massed together appear darker than in the resting cell. The aggregation continues and the entire nuclear substance appears darker, due to massing together in small quantities of its contents (Fig. 5). These small thread-like masses soon fuse into an almost continuous thin thread. During this process the nucleolus retains its color relations, and as the aggregation of the nuclear substance and the formation of the thread occur, the nucleolus comes to lie in more and more intimate relation with these. The nucleolus is related to the nuclear reticulum in such a way that the fibers penetrate its substance—(this becoming the more evident during the aggregation)—and not, according to my preparations, as Dr. Montgomery states, merely wound around its surface. The nucleolus is the centre around which the aggregation of nuclear substance, which leads to the formation of the thread, occurs.

Nucleolar Division.—This is a very common feature. The rounded nucleolus (Fig. 1) loses its form, becomes elongate (Fig. 2), then dumb-bell shaped and finally a constriction occurs and two equal nucleoli are to be seen (Fig. 3). But the interesting feature lies in the fact that this nucleolar division occurs and is often complete before the slightest trace of any nuclear aggregation occurs, *i. e.*, before any trace of a nuclear division (Fig. 4). Frequently, nucleolar division occurs during the aggregation. This can probably best be explained by regarding the two nucleoli as centers, around which aggregation occurs. There is apparently no regularity as to the exact time for nucleolar division.

The nuclear thread is now of a bluish crimson color. It is a thin, long, almost continuous thread, irregularly wound through the nucleus. It consists of linin material, with the more deeply stained chromatin granules disposed at intervals along its course. At one or at several points this thread can be seen to *dip* into the nucleolus (Fig. 6). This is not to be regarded either as an exceptional phenomenon or one difficult to see. Every preparation of the bean root, which shows division at all, shows many such figures (Figs. 7, 8, 9, 10). This relation is retained as long as any trace of a nucleolus is present. The nucleolus now begins to transfer its contents into the nuclear thread. During the early formation of this thread it seems to be in connection with the nucleolus at many points. But as the smaller masses unite to form the long, almost continuous thread, it is usual to see this thread dip into the nucleolus at one point, though it is not uncommon to see this relation at two or three points (Fig. 9). That this is an actual dipping in and not a mere superficial relation, can not only be plainly seen, but the staining relations next to be described prove that this must be the case. The thread being in connection with the nucleolus, ceases to absorb crimson stain, as the nucleolus transfers itself into the thread. This thread, as it receives the nucleolar material, becomes thicker, and we have finally a thick thread—the spirem. All stages of transference can be seen. First, there is a large bluish black nucleolus and a long bright bluish-crimson thread; the thread becomes darker, the bluish color becoming more predominant than the crimson, and then stages are reached when it is difficult to perceive the crimson at all. Finally we have the completed spirem stained just as was the nucleolus (Fig. 11). And this spirem can now be described as a thick bluish black, irregularly wound thread (Figs. 12 and 13), consisting of linin with more deeply stained chromatin granules placed at intervals along its course. As the nucleolus is gradually transferring its contents to the thread, it naturally becomes smaller, and when the spirem is complete, the nucleolus has disappeared. One of the last stages in which the spirem is almost complete, and the nucleolus therefore very small, is shown in Fig. 11. Such stages, as one would expect, are rather difficult to find, for the spirem thread becoming darker and thicker as the nucleolus becomes smaller, is apt to obscure the nucleolus from view. The spirem now divides transversely into the chromosomes.

Relation of Nucleolar Substance to the Material of the Spirem, or Chromosomes.

From the above observations, it can be stated that without doubt the chromosomes have derived at least a large part of their material from the nucleolus. As the spirem becomes more and more deeply stained, we know this staining relation to be due to the chromatin. When the thread first forms, it is of a bluish crimson tint and is formed from the nuclear substance. This nuclear substance contains but a very small quantity of chromatin, so that while the nuclear thread contains some chromatin derived from the nuclear material, the quantity thus obtained must be relatively small. The chromatin which eventually fills the thread and gives it its dense bluish black color is mainly nucleolar material. We have here merely a temporary change in form. The nucleolus is chromatin material, and the chromosomes are chromatin material. In the resting stage we find the chromatin chiefly massed together in this large bluish black nucleolus; in the dividing nucleus, the nucleolus is transferred from its aggregated condition and is distributed along the nuclear thread. Therefore, as the reserve chromatin mass, *i. e.*, the nucleolar substance is passing out into the nuclear thread, the nucleolus itself gradually disappears.

We can now proceed from the stage where the spirem has split transversely into the chromosomes. The nuclear membrane has already disappeared, it having started to disappear as the nucleolus passes its substance to the spirem. The chromosomes split longitudinally, and thus are formed the daughter chromosomes (Fig. 15). There are in most cases five daughter chromosomes. Four is not an unusual number to find, but five seems to be the more common. The daughter chromosomes now pass to opposite ends of the central spindle. They collect at the ends of the spindle and for a time retain their individuality (Fig. 16). Soon the individual chromosomes can no longer be distinguished, since they aggregate to form a small dense blue-black coil (Figs. 16 and 17).

Reappearance of the Nucleolus.—The chromosome coil now begins to heap up its chromatin material and we have the beginning of the formation of the nucleolus. The very earliest stages of its formation, as one would expect, are very difficult to find, for the deeply stained chromosomes obscure the first traces of the nucleolus. That the chromosomes employ their chromatin to form the nucleolus is easily demonstrable in slightly later stages. As the chromosome coil gives up its chromatin elements, it loses its dark color gradually. At first these chromosomes are full of the dark chromatin granules; these become fewer and fewer as the nucleolus becomes larger and more distinct. The chromosome coil or skein now appears crimson. Many stages can be found (Figs. 17, 18, 19), where a few deeply stained chromatin granules can still be seen in the thread.

Finally nearly all the chromatin is passed from the crimson thread to the nucleolus. The thread soon loses its evident connection with the nucleolus, and spreads out to form the nuclear reticulum. The nuclear membrane reappears during the time the nucleolus is being formed. When the nuclear membrane reappears, the first traces are always on the *outer* poles of the differentiating nucleus.

Often instead of forming one nucleolus, the chromosomes empty their contents so as to form two nucleoli (Fig. 19). The two nucleoli may fuse before the resting stage is reached or as perhaps more frequently occurs, they never fuse, but exist as two nucleoli in the resting cell.

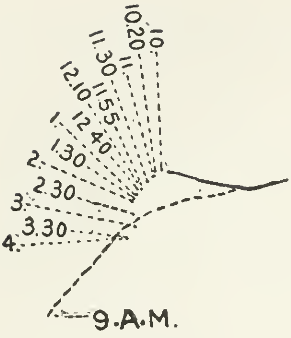
The daughter nuclei becoming complete, division of the cell is completed. This occurs by a pushing in of the protoplasm from the two opposite walls of the cell until these inpushings meet in the center. The spindle becomes fainter and gradually is lost. The division wall becomes distinct, the daughter nuclei perfectly formed and division is complete.

Function of the Nucleolus.—From the above observations it is impossible to regard the nucleolus as a nutritive centre. The nucleolus is, at least in large part, a mass of chromatin material, packed together in a rounded form, during the resting stage, and during this stage, it is the important chromatin centre. During division it becomes transformed into the chromosomes. The nuclear thread formed in the earliest stages of division merely serves as a path along which the nucleolus passes its chromatin. When the nucleus divides, it is necessary that its properties shall be distributed equally to the two resulting nuclei. This is accomplished by the splitting of the chromosomes ; the daughter chromosomes of each of the nuclei formed are equal in value.

Within recent years, the greatest importance has been attributed to the chromosomes as the *sole* bearers of the hereditary substance. It is true that the chromosomes are bearers of the hereditary substance. But no more importance should be assigned to these than is assigned to the nucleolus. The chromatin in the chromosomes contains the active hereditary substance, but in the resting stage, this hereditary substance is largely distributed or held in the nucleolus. The inactive chromatin elements of which the nucleolus is composed are the ones which become the active chromatin or hereditary substance of the chromosomes. Therefore we must assign to the nucleolus as well as to the chromosomes the function of containing hereditary substance. The only difference is that these hereditary elements are inactive in the nucleolus, whilst in the chromosomes, they are in an active state.

My best thanks are due to Dr. Mary Schively, who kindly reproduced the figures of the accompanying plate from my stained preparations.

PLATE I.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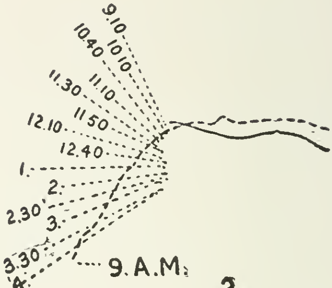
9.A.M.

1.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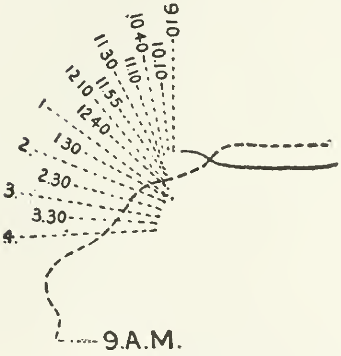
9.A.M.

2.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4.
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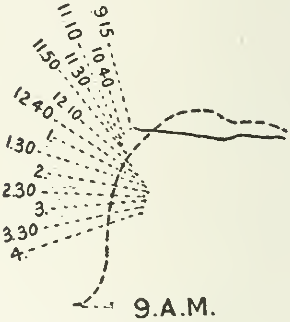
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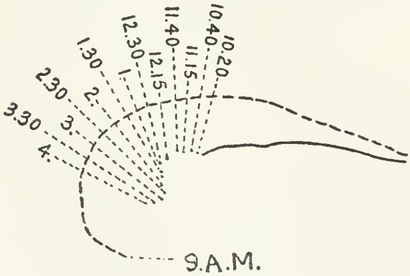
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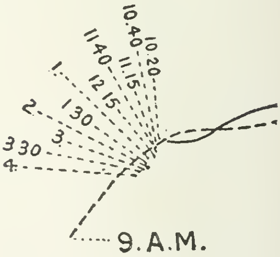
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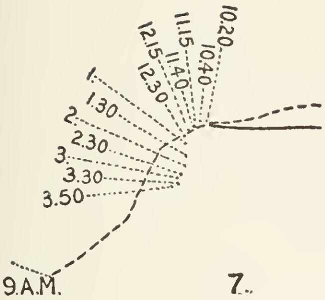
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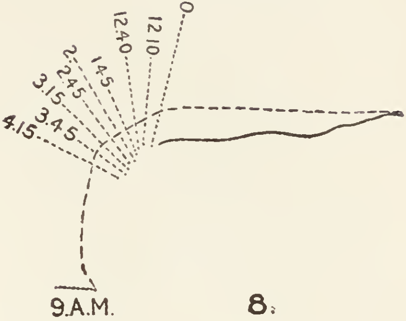
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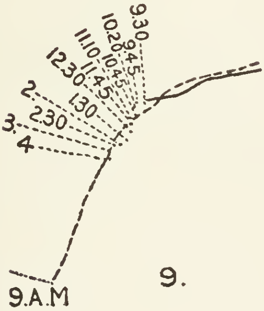
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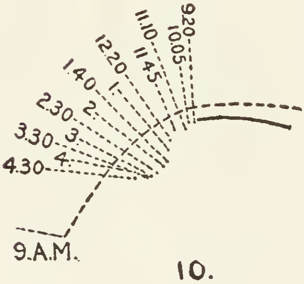
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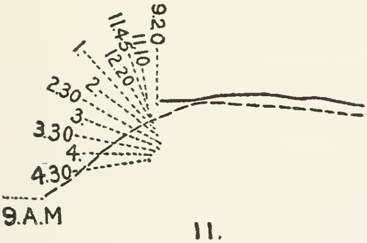
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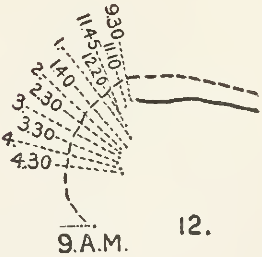
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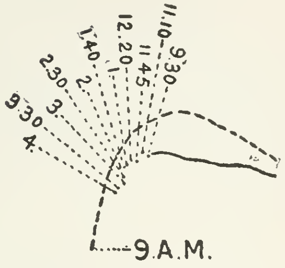
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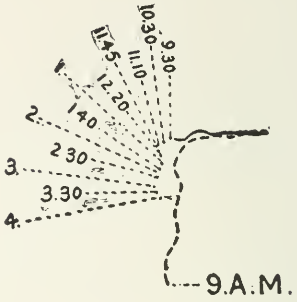
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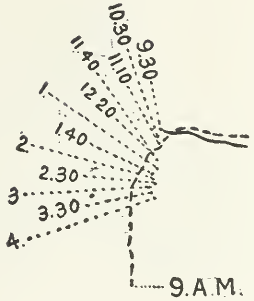
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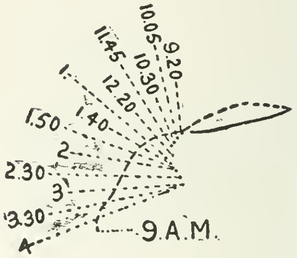
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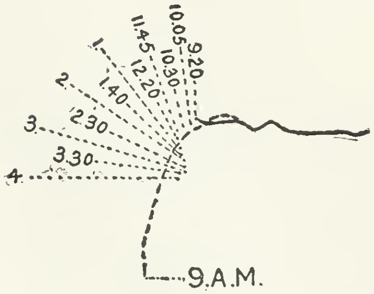
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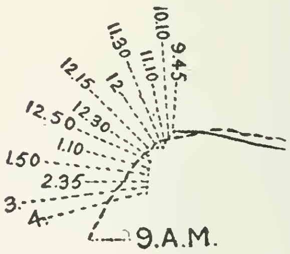
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Current Problems in Plant Cytology.

By J. M. Macfarlane.

[Presidential Address to the "Society for Plant Morphology and Physiology," delivered at Yale University, December 29, 1899.]

We are now met as a "Society for the Study of Plant Morphology and Physiology" on the threshold of the closing year of the nineteenth century. It is alike appropriate and helpful in human affairs that, at the close of each great period of endeavor, a brief survey should be made of the achievements and failures of the past, and that fresh plans be mapped out for higher achievements in the future. Every traveler through a new country seeks from time to time some vantage point, from, which to survey the ground already trod, and the newer regions that lie beyond. I trust that it may not seem inappropriate, if I claim the standpoint and vantage ground of the living cell as that from which we can best survey our domain of plant morphology and physiology. I have accordingly chosen as a suitable subject for the present occasion "Current Problems in Plant Cytology."

Who, at the beginning of this nineteenth century, accepting the measure of progress in past centuries as a gauge for the future, could have predicted that we should now be able to boast of so great and noble a heritage? Macroscopic morphology had then just emerged from the condition of a confused aggregation of facts and fancies, into that of a stable system of correlated principles, thanks to the efforts of Linnæus, Bernard de Jussieu and Gärtner. The beginnings of microscopic morphology had been laid by Malpighi, Grew and Casper Wolff, in the preceding centuries, though an inspection of their works graphically proves how diligent must have been the men, but how imperfect their methods for histological study. In plant physiology Linnæus, Koelreuter and Sprengel had advanced inquiries into the fertilization, pollination and hybridization of plants to a degree that excites our highest admiration. Stephen Hales, Ingen-houss and de Saussure had grappled with problems of plant nutrition. Investigations into plant irritability had been largely confined to naked-eye studies of the leaves of *Mimosa*, and the stamens of *Cynara*, *Opuntia* and *Berberis*. The important discovery by Corti in 1772, of protoplasmic circulation in *Chara*, was lost as a permanent contribution to science for half a century, through a false interpretation of the phenomena.

The dawn of 1800 brought, therefore, only moderate hopes for the century now closing. The splendid results achieved, by all the workers who have lived through its years, have been due to unswerving faith in the motto "To the solid ground of nature trusts the mind that builds for aye."

It would be equally difficult and invidious were I to compare minutely the work and the workers of the century. Rather let me attempt to sketch hastily the great advances which have been made directly or indirectly owing to our increasing knowledge of the cell as the plant unit. In the first quarter of the century numerous investigations were directed to the cell wall as a growing and a mature structure. Each investigator was thereby drawn more and more closely to the formative substance from which such walls proceeded, as furnishing the real basis for explanation of their growth, patterns and uses. In the second quarter of the century, therefore, the researches of Von Mohl, Schleiden and Robert Brown culminated in the recognition of the living cell as the plant unit. Botany thereby attained, for the first time in its history, to the dignity of an exact science. True, the organic units that make up each plant do not usually behave in the manner that chemists and physicists can predict for the simpler molecules with which they deal, but this, instead of detracting from Botany, should be accepted as evidence that the laws governing the life of every plant unit are the more complex and profound, and, therefore, the more worthy of closest study. Coincident with this advance were the studies of Naegeli and Brücke on cell structure and irritability, of Hofmeister on the evolutionary relation of cells and tissues in vascular plants, and of Herbert on hybridization.

The third quarter of the century ushers in a galaxy of giant workers, who produced epoch-making works, all founded on cellular morphology and physiology. That this period is marked by the appearance of Darwin's numerous contributions is ample testimony to its fruitfulness. The works of Berkeley, de Bary, Tulasne and Brefeld on the Fungi, of Schwendener on the Lichens, of Hofmeister on the vascular plants, and of Sachs on general physiology were rendered possible only by recognition of the cell as the fundamental unit of plant life. The advances of the quarter century that has now nearly run its course are known to all, but the outstanding feature of the period has been the remarkable increase in the number of workers who are contributing their share to the common stock of morphological and physiological knowledge. No previous quarter of a century in human history has equaled the present in the variety, wealth and novelty of results.

Let us attempt now to survey our present day knowledge, and inquire regarding lines of progress for the future. A proper conception of plant cytology at once brings before the mind groups of problems, any one of which already claims the attention of specialists from the multiplicity of details that bear on it. It may seem presumptuous, therefore, on my part to attempt to review and compare the studies which have already appeared, but every teacher of his science must accomplish this more or less perfectly. Though a somewhat arbitrary method, I may be permitted, as a matter of convenience, to glance at cell life from the standpoints of (a) morphological cytology, (b) physiological cytology, (c) experimental cytology, (d) ecological cytology, (e) evolutionary cytology, and (f) taxonomic cytology. As in all natural systems of classification no one of the above is sharply demarcated from another, so that in attempting to treat of each there must of necessity be an overlapping.

(a) MORPHOLOGICAL CYTOLOGY.—Account might here be taken not merely of the resting and dividing cell, but of many problems relating to food products. I propose merely to touch upon the structure of the active cell, and in this the living protoplasm claims first attention. Though considerable emphasis has been laid on differentiation into zones, it seems still a very doubtful question how far we should attempt to speak morphologically of ectoplasmic and endoplasmic layers. Appearances often strikingly suggest such. The creeping "feeling" colorless margins of a living Myxomycete, or the same when fixed and stained, contrast strongly with the highly granular enclosed mass, but the extremely fine motile layer of protoplasm, which circulates immediately within the cellulose membrane of *Spirogyra*, and which is so clearly observed when the cell is dividing, encloses and carries along many granules of varying size. Like diversity characterizes higher cell types. It seems premature, therefore, to attach too much importance as yet to structural differences in ecto- and endo-plasm. But in much of the recent literature on cell structure and karyokinesis there is a growing tendency to recognize a fine system of radiating, and at times interlacing, "kinoplasmic" threads traversing the protoplasm. These threads, according to some authors, are peculiar to it, or are continuous with certain threads of the nucleus, in the resting or dividing state according to others. Three years ago, before the American Society of Naturalists I spoke of a fine network, containing chromatin substance, as being present in various plant cells distributed through the protoplasm and continuous with enclosed nuclear chromatin. The existence of such a network has since been demonstrated in a variety of species, and in many instances the threads of it link together the plastids where such occur. From its bright refractive appearance, its evident continuity with the nuclear membrane, and its behavior to fixatives, to corroding agents and to chromatin stains, I would regard it as an extremly fine network, built up of linin and chromatin constituents, continuous with similar constituents of the nucleus.

But with Gardiner's work on intercellular protoplasmic continuity before us, an important though possibly difficult line of inquiry will be to ascertain whether the continuity be one of the general vegetative protoplasm only, or whether there does not exist an intercellular network of linin-chromatin fibres. In dividing cells of banana root, bean root, polygonum stem, etc., we can at times see in adjacent cells that are dividing, strands of a denser substance than the protoplasm, which appear to be continuous as longitudinal striæ from spindle pole to spindle pole. May we not therefore have an intercellular linin-chromatin network which may link together not merely the protoplasmic, but the hereditary substance? Townsend's researches on the apparent intimate relation between nucleus and cell wall suggest such a condition, while the wave-like rhythm of division-activity which traverses most filaments of *Spirogyra* during division, suggest similar relations. A careful study of the latter plant has given as yet only negative results.

Before considering the nucleus it may be pertinent to inquire as to the value to be placed on differential stains, and specially the erythrophil and cyanophil reactions. Since the studies by Schwartz and Rosen, so many conflicting views have been advanced that Miss Huie's observations on *Drosera* were specially welcome. Wager's observations on hymenomycetous cells prepare us to believe that the relative stainability of parts may be largely or wholly reversed during division, as do the later studies of Miss Sargant, Farmer, Wager and Dixon.

Miss Huie's experiments on the feeding of *Drosera* leaves prove that an intimate relation exists between the nucleus and nucleolus, and that reciprocal chemical changes are constantly proceeding in them. While differential stains may often aid us in the *demonstration* of structural details, they may prove fallacious guides in the *interpretation* of morphological and micro-chemical relations.

The *nuclear membrane* is now so universally conceded to be a morphological feature of the resting nucleus that the application of a definite name is appropriate. Apart from the study of living cells, carefully fixed material alone presents the natural appearances. Alike in living and stained cells it shows striking resemblances to the threads of the nuclear network internally, and to the radiating kinoplasmic threads which traverse the proptoplasm externally. In some plants it is evidently continuous with the latter. But where imperfect fixation has been effected, the membrane often resolves itself into a system of threads and knots, in no way different from the nuclear network. This may be even characteristic of living cells, as we shall have occasion to mention later, and has caused Wisselingh to describe the membrane as made up of "small bodies, lumps and granules." Its behavior when fresh cells of *Spirogyra nitida* are simultaneously swollen up and stained by 50 per cent alcoholic eosin is instructive. As osmosis proceeds, the radiating strands from the nucleus snap suddenly along one side, the enveloping protoplasmic mantle of the nucleus then immediately expands on that side, while the nuclear membrane remains as a puckered but continuous envelope. But it is during the dividing state of the cell that we can learn most as to its morphology. In *Spirogyra nitida*, during the prophase period, it persists for a considerable time as a spherical bladder, surrounded by a delicate protoplasmic sheath, and connected by refractive threads with the nucleolus internally, and by the radiating strands externally, while definite pores in it permit the outflow of the nucleoplasm. In other plants most observers describe its gradual absorption during the middle, or later prophase stages. This is clearly seen in dividing bean, corn and banana cells. The aggregating constituents of it, which have been stained by iron-hæmatoxylin and Bordeau red, indicate a linin and chromatin combination. In daughter cells which are passing from the anaphase to the telophase state, the nuclear membrane differentiates first round the outer ends of the poles, and gradually grows round the nuclear substance. This differentiation is contemporaneous with redistribution of the linin and chromatic substance, and reformation of the nucleolus. The recent statements by Wisselingh that the nuclear membrane, network, and nucleolus undergo varying degrees of corrosion, suggest specific physical or chemical differences, the exact value of which we cannot as yet estimate.

Nuclear Substance.—If we exclude the nucleolus, the nuclear substance with its membrane make up the bulk of the nucleus. Many histologists now distinguish three components in it, the nucleoplasm, the network or linin substance, and the deeply-stained granules or chromatin. Appealing again to dividing cells, the role of events consists in the aggregation of refractive substance of the nuclear membrane and still earlier of the network, and, as is being more and more fully recognized, with distribution in most cells of the nucleolar substance. When preparations are even slightly over-stained the aggregating substance is uniformly dense, but a proper degree of staining reveals that the aggregating loops or threads are made up of more deeply-stained bodies, the chromatin granules, as described by Debski and others, which are disposed along a rather less deeply-stained ribbon, the linin. As the prophase advances toward the metaphase stage, splitting of these granules occurs, as described carefully by the above author. Later, the longitudinal splitting of the ribbon along which these are disposed, gives rise to the daughter chromosomes.

The main point of discussion at the present time is as to the possible value of the three constituents just described. The nucleoplasm does not seem to differ fundamentally from the general cytoplasm. The nuclear framework, however, presents greater difficulties. During the past ten years or more, this has generally been regarded as composed of linin and chromatin constituents intimately related, the latter as the bearer of hereditary substance, the former as the framework for distribution of the hereditary substance. Great emphasis has been laid on relative stainability of these and of the nucleolus by acid and basic stains. But the past two years has witnessed a decided change of opinion by many authors. Fischer's recent definition of chromatin is: "The nucleic acid containing stainable substance of the cell nucleus, which with increasing content of nucleic acid always stains less strongly with acid watery stains."

Such a definition does not preclude the presence of substances which may be related to nucleic acid on the one hand, and to albumens on the other. It seems, in fact, as if the fundamental mistake of the past fifteen years has been in accepting that the highest phosphorus-containing portion of the nucleus, viz., chromatin, is the most important material as hereditary substance, and that all else was accessory to it. The nucleolus, as well as parts of the nucleus, consist of complex albuminous compounds. The linin substance stains deeply with many protoplasmic stains, and is large in amount. Undue emphasis we believe has been given to the chromatin granules as the sole bearers of heredity. Since linin and chromatin alike build up each chromosome, since both share equally in the division process, since both exist in spore and sex cells, and since both, according to my own studies, as well as those of Wisselingh and others, make up the stainable nucleolar substance, may we not regard the general cytoplasm and nucleoplasm as *the vegetative substance of the cell*, the chromatin granules as *the highly specialized hereditary substance*, and the linin as an intermediate substance which transmits hereditary peculiarities in slow degree from the cytoplasm to the chromatin, and is itself the bearer in part, of hereditary peculiarities. To this we can conveniently return after consideration of the nucleolus.

The literature bearing on the *nucleolus* has been so recently collected, and expanded from the zoological standpoint in Montgomery's valuable paper, that it may seem necessary only to compare the views there presented. But so rapid have been the advances on the botanical side during the past two years that the views of Wager, Debski, Farmer and Williams, Sargant, Wisselingh and others, might importantly modify our conceptions.

The resting nucleolus, when fixed and stained, shows a density of stainability that is only and exactly paralleled by the chromatin granules, and to a less extent by the linin. Moreover, the total amount of the latter substances in the perinucleolar area of most embryonic cells in the resting state, would not account for the bulk of the chromosome granules. On the other hand the vegetative cells of most coniferous plants, and in such growing apices as *Passiflora*, *Nerium*, *Bougainvillea*, the nucleolus, though distinct, is relatively small, while the perinucleolar area is rich in refractive stainable substance in the form of loops. As is now generally acknowledged, the resting and dividing nucleolus of species of *Spirogyra* bears nearly all the chromatin as well as linin material, and in the dividing stage becomes halved, when the halves retreat to opposite poles of the nuclear spindle. The growing importance attached to the nucleolus as a result of recent research, tends to confirm the opinion that it may yet be regarded as the most specialized portion of the cell. The recent paper by Dixon contains many good arguments in favor of such a position. It is frequently objected that the nucleolus is a homogeneous mass of formless character, but though its extreme density often suggests this, detailed study reveals minute structural dissimilarities. Further, I have frequently observed during the earlier prophase stages of many cells that the aggregating chromosome substance dips into and fuses with the nucleolar substance, and that both contain elements which stain with equal intensity. This is to some degree confirmed by Farmer and Sargant.

While only one nucleolus seems typically to exist in embryonic cells during the prophase stage of division I have so often observed two, at some distance apart, that their division, as inaugurating division of the nucleus, is strongly suggested. Whether during the metaphase in plants other than *Spirogyra*, there is a sac-like remnant of the nucleolus left, which divides and in time receives the material that has been distributed along the chromosome, is an unsettled question. In the early anaphase, however, one can generally see deeply staining daughter nucleoli within the nuclei.

Since the nucleolus behaves chemically and to stains like a nucleo-albumin, since this is more complex than the nucleic acid of chromatin substance, since the nucleolus is the main center of division activity in *Spirogyra*, and since in many embryonic cells it forms the largest mass of stainable material, and is converted into the bulk of the chromosomes, its prime importance should be conceded. The thought seems to be behind much of our current cytological literature, that it more than the nuclear substance dissolves or loses its identity.

This, I think, is a mistaken view. Both exhibit an equal degree of plasticity.

With the evidence now before us, I would suggest that every cell consists of the general protoplasm and of the nucleoplasm, these together constituting the *vegetative substance of the cell*, while the linin and chromatin or chromosome substance constitute *the hereditary substance of the cell.* In many cells the latter is chiefly aggregated in the nucleolus during the resting phase. From the nucleolus threads radiate out through the nuclear area and fuse to form the nuclear membrane, while from it radiating prolongations traverse the protoplasm. Whether such threads are continuous from cell to cell through pores in the common walls is a question still needing solution.

The Endonucleolus.—At a time when the nucleolus was regarded as of small moment, I noticed as a uniform feature within it, in embryonic cells, a small clear body which was named the endonucleolus. Its constant presence in a wide range of tissues warranted me in regarding it as an element of possible importance in cell life. In recent years it has generally been spoken of as the nucleolar vacuole, and has been viewed as an inconstant factor. Meunier's statement that it is absent in living cells of *Spirogyra* is unquestionably erroneous, though owing to the great density of the nucleolus it is frequently difficult of detection. If we are content to call it a vacuole, this will depend on the meaning which may attach to the term. That it is often overlooked in embryonic tissues is to be explained by the overstained state of preparations. I would still insist on its general occurrence and probable importance in living cells. Montgomery (p. 508) says: "In opposition to Meunier, and in agreement with most investigators, I must conclude that vacuoles are normal structures in nucleoli, since they may be seen after the most diverse methods of fixation, and their size and number are not only to some extent limited for the particular cell, but are also different at different periods in the metamorphoses of the nucleus. It is the rule that the youngest nucleoli are homogeneous, and that vacuoles first arise when they have increased in size. Their size and number vary at different phases in the development of the nucleolus. Very frequently a number of smaller ones appear, and then these subsequently fuse together and produce a larger one." The first statement in the above quotation I would endorse, but must dissent from the remainder, as being opposed to all conditions in *vegetable* tissues that I have examined. A small endonucleolus is seen alike in living and stained nucleoli in average embryonic cells. With advancing age of the cell the size and number of endonucleoli increase. It must be conceded that when viewed within the dense substance of the nucleolus, the endonucleolus possesses a rather vacuolar aspect, and it is probably true that its function is to store some special liquid for the nucleolus. We cannot as yet speak definitely as to its constitution, but I would suggest that it possibly plays a special part in furnishing to the nucleolar substance some ferment or compound which may be utilized during the division period. To treat such bodies as haphazard vacuoles filled with cell sap will not conduce to a better understanding of them.

As regards that most attractive of all cell elements the centrosome I do not consider that we have evidence on which to base a safe conclusion. Strasburger's excellent review of the position in his recently published work leaves us largely in doubt, but shows that much painstaking study has still to be engaged in.

(b) PHYSIOLOGICAL CYTOLOGY.—Though it would be impossible arbitrarily to separate the two, I venture to affirm that great though the problems of morphological cytology have been, these will be equaled, if not eclipsed, in the near future by problems of a more purely physiological kind, while these again may ultimately be surpassed by physico-chemical results. Meanwhile it is needless for us to attempt reaching the last hastily, except by the bridge which function has built for us. No function in animals is of more prime importance than that concerned with the taking up and propagation of external stimuli. Through it has been called forth the complicated nervous system, which brings each animal into proper relation to its environment, and enables it to react on this. The investigations into plants along similar lines are still comparatively few, but largely owing to the simpler constitution of plants, the phenomena of irritability can be more easily studied.

We now know that not merely such receptive centers as the pulvini of sensitive plants, the tentacular knobs of *Drosera*, and the irritant hairs of *Dionæa* are irritable, and can also propagate a stimulus, but that stimuli can equally be transmitted from apparently passive parts. Thus a stimulus can be received and propagated from any part of a leaflet of *Mimosa*, or even from one of its stipules, from a cup gland on the petiolar base of *Cassia nictitans* and its allies, or from any part of the leaf surface of *Drosera* and *Dionæa*. What the substance or substances are which conduct such stimuli, and what the molecular changes are that are produced in these, we are still largely ignorant of, but that such primarily reside in, or are governed by protoplasm, appears to me to be still as true as when Sachs wrote: "We can at present form no idea why this change in the protoplasm occurs in consequence of a stimulus, and with what molecular changes it is connected; it must suffice for us meanwhile to know that the externally perceptible effects of stimulations are caused by the change referred to in the protoplasm itself."

The specific irritability of such sensitive plants as *Mimosa pudica*, *M. latispina*, *Schrankia uncinata* and *Desmodium canescens*, gives to each different latent periods, times of propagation of stimulus, varying summations of stimuli, and recovery periods, that are constant for each under the same environmental conditions. These specific variations must be referred ultimately to specific cytological differences in reaction of the living substance.

Again, in the same group of plants, the cells with "aggregation" contents that make up the bulk of the irrito-contractile centers or pulvini are still very imperfectly understood, but the comparatively sluggish manner in which each aggregation mass collects in *Drosera* tentacle or the pulvinus of the Tick Trefoil, as compared with the lightning-like rapidity shown in cells of *Mimosa* pulvinus indicate specific cytological differences that are as interesting as they are profound. The nature and function of the crystal cells that surround the bundles, and of the special cells of Haberlandt require additional elucidation.

Though it is from the botanical side that our knowledge of the tropisms first originated, we have still to determine accurately the cytological changes that accompany or originate these. The experimental fact recorded by Dr. Schively that lateral shoots of *Amphicarpæa*, which are strongly geotropic, may become apogeotropic through no direct action upon them, but from removal of the neighboring main axis above the point of insertion of the branch, points to some fundamental alteration of the cell substance, possibly in relation to nutrition. The reversal of geotropic response in the peduncle of *Tussilago*, and of the heliotropic response in the peduncle of *Linaria Cymbalaria* are similar growth-reactions whose cytological analysis will be of the highest interest. The conclusive demonstration by Pfeffer and Correns of the localization of geotropic irritability in the embryonic cells of the root tip may either indicate a weakening or loss of functional activity in the cells as they become older—resembling the decreasing irritability in older leaflets of *Robinia* or *Cassia*, or this may be due to the retention in the cells of the growing apex of some special protoplasmic compound.

But the close of this century has given us some valuable illustrations of chemical relations between living cells which a few decades earlier would have been regarded as physiological. The chemotactic relation of sperm cells in the mosses and ferns to definite chemical substances excreted by the egg apparatus opens up a wide avenue for research that will not be confined to fertilization phenomena alone. The remarkable constancy in position, of the main mass of hyphæ of symbiotic fungi in the medio-cortex cells, in roots of orchidaceous, liliaceous, burmanniaceous and many dicotyledonous plants, suggests as pointed out by Groom, the presence of some chemotactically active product, for which the hyphæ have an affinity. These are a few of the first gleanings in a great harvest of chemico-physiological generalizations that will doubtless be reaped in the century soon to begin.

(c) EVOLUTIONARY CYTOLOGY.—Since the formulation of the evolution hypothesis forty years ago, much has been written on the factors of organic evolution. Many of the observations have been direct and valuable contributions to cytological literature. But while such inquiries as "the possibility of characters being acquired," or "the hereditary transmission of acquired characters," or "the action of environment" have been debated almost *ad nauseam*, the inquiry has not been followed out in most cases, as an exact line of cytological investigation, the results of which would aid us in determining what are the characters latent or observable which every cell or cell-group possesses.

With his usual sagacity Darwin constantly insisted on the recognition of latent structures and functions as important factors in the life of organisms. Molecular details which may be entirely hidden even to our aided view, or subtle functions which definite molecular combinations may call forth, might alike be overlooked by us, and yet prove potent agents during the life rôle of organisms. Much of the serum therapeutic treatment of animal diseases at the present day is based on such a view, for whether a diphtheritic antitoxin or an antivenin, both seem to be substances whose chemical activities are called forth by the presence of an antagonistic compound that is injurious to living tissues.

Accepting it that the higher plants have been evolved from simple types, it might be inferred from much of the current discussion that many important substances have been acquired during the process of evolution. To estimate the truth of this we might attempt to compare the stock in trade, so to speak, of unicellular plants as existing now, with that of the highest multicellular plants. We are immediately struck with the remarkable uniformity in both. Cellulose and mucilaginous walls that grow by apposition, the living constituents that are enclosed, the varieties of plastid appearing in the protoplasm, the carbohydrates, amides, proteids and other food constituents, the numerous ferments admirably presented in Green's recent work, the acids, alkaloids, pigments, and even crystals, are common property of both ends of the series. True, we do not find all of these located in any one species, but they may nevertheless be viewed as one common heritage throughout the entire plant scale. With our present imperfect knowledge, it would be rash to dogmatize as to which of these some plant species has or has not the active or latent power of producing, when the appropriate stimulus is applied. If two related species or varieties agree in all points, except that one appears red, while the other is green, the red pigment might fairly be viewed as a new formation, while in reality it may have appeared in previous generations of the plant, and the latent machinery for its development may have persisted. Later I will refer to some striking cases of this kind which have come under my notice. In what then does evolution consist? If in passing from the simple to the complex plants no *great* variety of new primary constituents is observed, but rather an advantageous placing of those already existing to suit environmental conditions, evolution might be supposed to consist mainly in an increasing distributional adaptation of cell substances, some of which may at times be well developed, while others may remain latent, though the complex machinery in the latter case would be continued, ready to start the formation of temporarily undeveloped products when appropriate external stimuli are applied.

An important inquiry of the future then should be to ascertain minutely what cell substances, if any, are peculiar to the higher plants, to take accurate note of the occurrence, or the appearance and disappearance of elaborated products in related species, and to determine whether by altered environment products that seem for a time lost in the chemistry of the cell, may not reappear under altered sets or strengths of stimuli.

We must therefore recognize, as a line of cytological inquiry akin to the last, but somewhat different in its method, my next topic, viz :

(d) Experimental Cytology.—If plants can change under altered environmental conditions, such changes must first occur in cells or cell groups. By experimental methods often of a simple kind, wonderful insight has already been got into cytological adaptability to environmental stimuli. Without lingering over the old experiments on the thickening and strengthening of tendril tissues, when these successfully wind around other bodies, and their atrophy when the latter are wanting, we have recently got many suggestive thoughts from the writings of Bonnier, Henslow, Lazniewski, Goebel, Lothelier and quite recently of Teodoresco. Their studies on lowland and alpine plants, on inland and littoral plants, on xerophytic and hydrophytic plants, or on the response of plants to different light rays have opened up new possibilities for experimental work. I may be pardoned if I refer to a few which have come under my personal observation. Experiments performed by Dr. Schively on fruits of the hog peanut conclusively prove that strong lignification of the cells after a definite pattern will take place in two distinct zones of the carpels when the fruits are exposed to the air, while the same cells will remain thin and delicate if retained in moist soil. Whether moisture alone, or moisture and darkness combined, produces this effect has not yet been determined.

Several years ago my attention was arrested by the apparent varying behavior of our native Sarracenias to varying degrees of illumination. When the common species—*S. purpurea*—grows under full exposure to the sun's rays, it is of a deep crimson color. This is due to the presence of a crimson pigment in the sap of the epidermal cells. When slightly shaded by low herbage in an otherwise open situation, it is crimson green, but lined along the veins with a deep purple color. When shaded by shrubbery for several hours daily it is green, streaked by narrow purple lines, and finally, when under a continuous shade, it is uniformly green. By removing plants of each of these to situations where the degree of illumination can be adjusted, I have proved that pigment production is a latent, or feebly expressed, or well developed quality of the epidermal cells, according to the intensity of environmental light stimuli. Even more interesting is our common southern species, *S. flava*. It is nearly always of a bright green or a yellowish green color in its pitchers, though about half the individuals of a meadow will show a few rich crimson lines along the back of the throat. The latter when grown during successive seasons in shaded situations will develop only green pitchers. But rarely over wide stretches of territory in South Carolina and Georgia, specimens may be gathered that are as richly colored as the finest of *S. purpurea*. When such are removed and experimented with, the coloration effects during succeeding years can be made to vary according to the degree of illumination. Least pigmented of all the native species is *S. variolaris*, which, in the shade, is a pale green, but in hot sunny meadows may show a reddish yellow flush. Historically it may be noted that John Bartram recorded similar color relation in wild plants of *Dionæa.* My experiments on this plant prove, that the deep crimson pigment, developed in the cells of the leaf glands, equally responds to environmental treatment as do the Sarracenias.

But similar results may be obtained from totally different environmental stimuli. Botanists who have only seen *Mimosa pudica* grown under glass with direct insolation, and at a temperature of 30°–35° C. might on first glance fail to recognize an individual of the species that had grown for some weeks in the open, during spring or autumn. Exposure to cold night winds causes the stems, petioles and midribs to assume a brick red hue that gives a new character to the plants. On a large scale we see the same change annually produced, when acres of *Gualtheria, Kalmia angustifolia, Cassandra*, and many other perennially winter-green plants are exposed to the drying winds and cold temperatures of winter.

Experiments even which may appear unnatural, often throw a flood of light on cytological questions. Thus Townsend's experiments on lacerated cells and those of Mottier on the effects of centrifugal force, though in themselves unlikely to occur in nature on a large scale, suggest points as to the relative density, resistance, and vitality of cells or cell parts which are of highest import. A perusal of Daniel's successive papers on graft unions reveals a healing and regenerative capacity of cells that is often paralleled in nature by the healing surfaces of stems and roots. But Daniel's work brings the student of cytology face to face with the deepest problems of heredity;

with the possibilities of protoplasmic fusions and transfusions, as well as nutritive adaptability.

No fact impressed me more in the study of *Cytisus Adami* as a probable graft hybrid, than the remarkable differences in the epidermal nuclei of the composite organism. While those of the arborescent *C. Laburnum* portion are relatively small and rich in chromatin material, those of the smaller parent portion, *C. purpureus* and of the hybrid, are large and much less refractive in their nuclei.

At the present day the complaint is sometimes made that plant taxonomy is being neglected for the cultivation of plant morphology and physiology. In one sense the complaint is a just one. But is it not true that we are only beginning to realize what plant individuals, plant varieties and plant species are, as we compare cell with cell, and tissue mass with tissue mass? That this is correct is evidenced by the rapidly growing popularity of that most recent departure which I will now shortly touch on, namely:

(e) ECOLOGICAL CYTOLOGY.—That cells, and therefore plant parts, can be molded by their surroundings, we accept as proved by experiment. Shortly, it may be said that environmental stimuli act on every living plant cell, while the cell reacts temporarily or permanently to the stimulus. This fundamental law of plant life is only beginning to be realized in all its fullness. But while the general relationship of plants to their surroundings in respect of form, consistence, size and color should be noted, it soon becomes evident that these data have to be corrected and supplemented by a study of individual cells. It is a significant omen of present day progress that Schimper's splendid work on plant distribution recognizes continually not merely those agents which we class as the environmental stimulants, but equally the cytological changes which such stimulants produce. So exact is this line of inquiry becoming that we may soon be able to speak not only of the morphological but equally of the physiological specific characters of plants. In my study of the great physiological group which we commonly speak of as "sensitive plants," I have been impressed by the evident specific physiological relation of the tissues to environmental conditions. In cold countries such plants can scarcely be said to exist, except in the person of the Wood Sorrel, in milder regions they begin to attract some attention, in sub-tropical countries they are pretty frequent, and in tropical regions they often give a character to many landscapes. But the increase in number of sensitive species toward the Tropics is correlated with increased sensitivity, so that from the Wood Sorrel to the Sensitive Plant we can arrange a graded series which show on stimulation a shorter latent period, increasing capacity for propagation of stimulus, a quickened period of contraction, as well as reduced neutral period, and period of re-expansion.

Bonnier's beautiful investigations on lowland and alpine plants should furnish a model for many similar studies in the future. The modifications he traces in epidermal cuticularization, in chlorophylloid cells, in tracheidal tissue and other bundle elements, demonstrate the true morphological origins of our Alpine Flora. Such considerations inevitably lead us to inquire—What is a species? Instead of attempting a direct answer allow me, in a few lines, to speak of what I have ventured to call "taxonomic cytology."

(f) Taxonomic Cytology.—Even if one should speak with bated breath of things taxonomic, before a Society for Plant Morphology and Physiology, at the risk of being considered a law-breaker, I will attempt to slip in the greater adjectival subject, under cover of the lesser substantive plea. Urgency seems to furnish the apology. During the past dozen years or thereby, as questions of heredity, transmission of character, and validity of species distinction have been debated, the necessity for some critical standard of reckoning has doubtless been painfully felt. The possible transmission of characters has been debated, but the vaguest views have been expressed as to what such characters are. Or again, species have been described as new which differed only from some older type in the size or folding of some part, in its relative hairiness, in its prostrate or upright habit, or in certain color effects.

One great occupation in the coming century will undoubtedly be, the elucidation of the precise morphological details of varieties and species, as being the only correct guide to the evolutionary affinities of our plant groups, and the segregation of species within these. All this must be accomplished from the standpoint of the cell as the ultimate factor. Whether in the upbuilding of unicellular or multicellular hairs of definite structure, in the presence of definite pigments over certain areas, in the development of thickening zones and other structural features, precise information is only obtained when such are recorded in terms of cell life. It will then be possible to classify plants on a natural plan, instead of as now by selecting one or two points of resemblance—not necessarily of morphological contact—as a taxonomic basis.

When such data shall have accumulated, the student of plant evolution will be in a position to compare varieties and species morphologically, to inquire intelligently how these are related to their environment, and to determine the limits within which variation may take place in a given time or under given conditions. The small beginnings have already been made, the coming century will doubtless witness the great continuations. Problems of surpassing interest invite our attention. My earnest hope is that our Society shall aid in the solution of some of them, and that annual contributions of ever-increasing value will be made to its Proceedings.

Fasciation in the Sweet Potato.

(WITH PLATE XIX.)

By Henry S. Conard, A. M.

The occurrence of fasciation or broadening of normally round plant axes has been often noted in a great variety of plants. Ferns, lycopods (9), gymnosperms, monocotyls and dicotyls alike show it, in frequency according to the order of mention ; it is most commonly observed in dicotyls. In shrubs, trees and herbs, following the increasing order, it has been recorded. The condition is most common in vegetative and flowering stems, being very rarely found in roots (1, 3, 7). It appears most in plants subjected to conditions of nourishment above the normal (4), occasionally, perhaps, as a result of disease (13) or injury (2, 8, 16). Frequently many of the plants in a certain locality will be fasciated, *e. g.*, a field near Hainesport, N. J., which yielded in the summer of 1899 dozens or even hundreds of fasciations of *Rudbeckia hirta* L. (11); also a meadow near Haddonfield, N. J., where *Ranunculus bulbosus* L. was quite as largely fasciated in 1893 (10); at Cape May, N. J., *Desmodium ciliare* DC. was frequently found fasciated by Professor Macfarlane in 1899; and, finally, at Fallsington, Pa., the writer observed fasciation in a species of *Lactuca* in 1899 and 1900. Such localities have been noticed to produce fasciations year after year. This fact, and the occurrence of so many individuals in so narrow a space, may result from the hereditary nature of this condition, as shown in *Celosia cristata* of gardens, and in de Vries' fasciated races. The Clyde strawberry bears fasciated flower-stalks, and hence, fasciated berries of huge size.

The common sweet potato (*Ipomœa Batatas* Poir.) as grown about Philadelphia produces fasciated vines very plentifully, and the same has been reported to me from Ohio, Kansas, North Carolina and Florida. Francis Windle, of West Chester, Pa., tells me that he saw fasciations on the sweet potato forty-five years ago. This plant, therefore, may be truly said to present another fasciated race. Among about two hundred widely differing species reported as being at times fasciated, the sweet potato seems to have been first recorded in 1897 by my preceptor, Professor J. M. Macfarlane, though observed by him in New Jersey in 1891. I may state here that this study was taken up at his suggestion, and prosecuted under his generous care and direction.

So far as man's influence is concerned, the sweet potato may be called an accidentally fasciated race, as opposed to the selected races of *Celosia*, *Crepis*, etc. For the conditions fixing this character in the plant have been simply those of high culture. Selection is, in this climate, carried on only with regard to quality and quantity of the edible roots. In opposition to this, I am aware that in tropical or sub-tropical regions the sweet potato is largely propagated from the growing stems of the previous crop. In this case the most vigorous vines might be selected each time, and these would probably be the fasciated ones, since they are so stout and densely leafy; thus fasciation might have been bred in by selection; but on this point no further evidence is at present forthcoming. My friend, Charles Barton, of Marlton, N. J., a considerable grower of "sweets," suggests that an excess of nitrogenous fertilizer (ammonia) in the soil seems to increase the amount of fasciation. At any rate, the evidence seems strong in favor of the view that fasciation in this plant is connected with high nutrition.

Passing now to more special considerations, we would offer the following observations, from notes taken chiefly on the farm of Charles Barton. The normal vine of sweet potato is from two or three to ten or twelve feet long, round, one-eighth to three-sixteenths of an inch in diameter, prostrate and rooting freely, green, with leaves arranged in two-fifths order. Five to twenty such runners come from one root. The longer vines, however, usually show more or less fasciation at their tips; they vary from a slightly oval cross-section to great bands two or three inches wide and about an eighth of an inch thick, bearing a dense head of leaves. Less commonly the shorter branches are fasciated, and occasionally a flattened sprout comes up in the propagating beds direct from the fleshy root. As a rule, even the broadest fasciated stem will be found round and normal near the parent root, and for three or four feet therefrom. Then, tracing toward the tip, the stem becomes oval and the phyllotaxy loses regularity; these conditions increase, one diameter remaining unchanged while the diameter at right angles to it increases until the malformation is fully developed, and the leaves are crowded together without any trace of order. The internal structure of fasciated stems is the same as in normal stems, modified only by the shape of the cross-section.

The frequency of fasciation in the sweet potato is shown by the following table, giving the condition in five plants taken at random:

	Number of Branches.	Length of Branch.	Character of Branch.
I.	2	9 feet.	Normal.
	1	6 feet.	Fasciated and crumpled.
	1	6 feet.	Phyllotaxy irregular.
	1	5 feet.	Normal.
	4	6–12 inches.	Normal.
II.	4	8 feet.	Fasciated at tips.
	6	Less than 8 feet.	Normal.
III.	1	. . . . .	Fasciated.
	5	. . . . .	Normal.
IV.	2	. . . . .	Fasciated.
	17	. . . . .	Normal.
V.	2	. . . . .	Fasciated.
	9	. . . . .	Normal.

I have yet to find plants of sweet potato entirely free from fasciation, though they have been carefully looked for; doubtless, however, many such exist. On counting the stems just as they lay on the ground, I found, in a poor location as to soil and exposure, 12 per cent of them abnormal, in good rich soil 18 per cent. Counting the tips or apices just as they came on poor and good soil, the former gave 20 per cent, the latter 54 per cent of abnormal growths. The stems show a smaller percentage, because even the fasciated ones are normal in appearance toward their bases.

Ring-fasciation.—Along with the ordinary fasciations there appear in the sweet potato, as in other fasciated races, various peculiar malformations, such as split or dichotomous branching, split fasciations, and especially that remarkable condition which has been termed "ring-fasciation." Of this last I would make especial mention, as it occurs in about one-half of one per cent of the abnormal stems.

The ring-fasciated stem is, like the flat fasciated, perfectly normal in appearance toward the root. Then, tracing it outward, it increases in diameter to three or four times the normal (one-half inch more or less), its leaves become irregularly placed, and finally the rounded growing apex is seen to be a hollow ring, instead of a knob of embryonic tissue (Fig. 6). In short, the ring-fasciation is a hollow stem, open at the growing tip. The hollow portion may include as much as two or three feet of the terminal part of a branch, and the cavity may reach a quarter of an inch in diameter. On splitting open such a hollow stem, small leaves and adventitious roots are found in the cavity. These leaves develop acropetally, those farthest from the open apex being oldest; but of course they never attain to any considerable size, nor can they become actively functional. They are more numerous toward the stem tip than lower down. In the case of cuttings planted in earth, roots spring from the inside as well as from the outside of the tubular piece, both passing down into the soil and becoming functional.

Histology of Normal and of Ring-fasciated Shoots.—For a better understanding of the cross-section of the ring-fasciated stem, we may first review the structure of the normal stem (Fig. 2). There is a cutinized epidermis bearing stomata, nectar glands[1] and pedicellate hairs; internal to it is the green cortex, bounded on its inner face by a distinct starch-bearing bundle-sheath. Within this is a ring of bast fibres, then true phlœm, cambium, xylem with many confused and insignificant medullary rays, and an unbroken pith. Numerous patches of internal phlœm that constitute a bicollateral bundle system lie between the xylem and the pith, as is frequently the case in Convolvulaceæ and allied orders. Milk canals are numerous in pith and cortex.

The ring-fasciated stem shows a doubling of the above structure, in that the internal cavity is surrounded by epidermis, cortex, and a fibro-vascular ring in addition to those forming the normal external wall of the stem. We find, therefore, in cross-section of the anomalous stem, the following zones, passing from without inward (Fig. 4): epidermis (*Ep.*) with cuticle, nectar-glands and pedicellate hairs, cortex (*Co.*), bundle-sheath (*B. S.*), bast fibre ring (*B. F.*), normal phlœm (*Ph.*), cambium (*Ca.*), xylem (*Xy.*), internal phlœm (*I. Ph.*), all exactly as in the normal stem (Compare Fig. 2). The pith (*Med.*) forms a ring separating these from the anomalous structures, consisting of internal phlœm of the internal or anomalous system (*i. ph.*), xylem of the internal system (*xy.*), cambium (*ca.*), normal phlœm of the internal system (*ph.*), bast fibre ring (*b. f.*), bundle sheath (*b. s.*), cortex (*co.*), epidermis (*ep.*), and finally the internal cavity (Compare Fig. 1).

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[1] Poulsen (18) describes nectar glands situated in pits at the top of the petiole of "*Batatas edulis*" (= *Ipomœa Batatas* Poir.), but makes no mention of similar, though less specialized, glandular hairs which occur on the lamina and on the stem of the plant. They are identical in structure and development with those described and figured by Ewart (6) for *Ipomœa paniculata*. They are so plentiful on the apex of the stem and on the young leaf rudiments as to cover fully seven-eighths of the surface of those parts. As the parts grow older the glandular hairs become brown and shrivelled, and most of them fall off. On mature leaves they were found beset with a fungus, as also were the petiolar nectaries; the parasite was evidently feeding on the nectar. Poulsen says ants and aphides are likewise attracted to the sweet potato tips; the former doubtless serve as a protective bodyguard against caterpillars, grasshoppers, snails, and the like.

The internal and external systems are in no wise different, save that the inner one is more slender and is inversely oriented. The internal epidermis bears stomata, nectar glands and pedicellate hairs like those of the external epidermis. The internal cortex is rich in chlorophyll as is the outer. The bundle sheaths of both systems are evident, their cells being filled with starch. The bast fibre rings are one or two cells deep in both systems. The xylem frequently has large tracheids in the cambial region. Both rings of cortex, and the common pith have numerous longitudinal milk canals.

Tracing the two tissue systems to the ring-shaped growing apex, we find both merging gradually and equally into the circular mass of meristem tissue. Leaf rudiments seem as plentiful at the apex on the inner as on the outer surface of the tube. The end of the hollow stem is not evenly truncated in outline, but has a wavy margin, or, more often, is split on one side, so that the stem continues as a plain or wrinkled fasciation (Fig. 5).

Following the hollow in the direction of the parent axis, the cavity becomes gradually narrower, until there is only room for one or two hairs to lie longitudinally in it. Then the faces of the epidermis come into contact, and finally this tissue ceases entirely, *i. e.*, the cavity is closed. Below this point then, we have the internal system reduced to a cylinder of fibro-vascular bundles in the center of the pith of a solid stem. Proceeding farther toward the root, the cylinder breaks up into separate bundles by dwindling away of the weaker ones between, and an irregular, interrupted ring of five or six bundles remains. These narrow down one by one until the xylem is represented by a single spiral trachea (Fig. 3). This trachea is surrounded on all sides by a zone of angular, radially arranged, phlœm—or phlœm-like cells; on the outer or centrifugal side this zone is five or six cells thick, on the inner side one or two cells thick. In longitudinal section the spiral trachea is accompanied by numerous sieve tubes and companion cells. Proceeding basipetally, the trachea disappears entirely; a little farther on, the sieve tubes give place to two or three pro-cambioid cells, which in turn are followed by small parenchymatous cells, and finally by ordinary pith. This transition takes place in the length of half an inch from the end of the spiral trachea. Thus the normal stem structure is reached, though this may be at a distance of two to five or six feet from the root of the plant. At no point do the vascular tissues of the two systems connect, though they merge into a common circular meristem tissue at the apex of the stem, where neither is yet differentiated.

Hitherto there have been described two undoubted cases of ring-fasciation, and two doubtful ones. In 1891, H. de Vries (5) described a spadix of *Peperomia maculosa* 30 cm. high, of which the upper 15 cm. were hollow. The lower solid portion was 0.7 cm. in diameter, spreading to 2 cm. at the top of the tube, where it was split into four lacineæ of differing size and length. The hollow spadix was covered inside and out with flowers, which lay in both cases above their bracts, showing that the inner development, like the outer, was acropetal. There were two close rings of distinct fibrovascular bundles, one on either side of a ring or hollow cylinder of pith, as we have just described in the sweet potato. These bundle systems were entirely separate throughout. The inner system, with phlœm facing the cavity of the stem became weaker below, some of the bundles, the writer states, changing their orientation of phlœm and xylem and continuing in the lower normal portion of the spadix as ordinary medullary bundles. Since in the sweet potato and in the case next to be considered, the internal bundle system vanishes entirely, it is especially curious to find some of its members in *Peperomia* becoming parts of the normal structure. The medullary bundles found in this plant are not present in the other two. This makes the investigation of *Peperomia* more difficult, so that it seems reasonable to suggest that even here the internal system may have vanished, some of the medullary bundles being distributed inward into the space thus left vacant.

The second case of ring-fasciation also came from de Vries, whose careful investigations have most contributed to make teratology a scientific study. The plant was *Veronica longifolia.* Fasciated material of this species was sent to de Vries in 1887, and by skillful propagation and selection the stock yielded in 1893 several hundreds of fasciated flower-trusses; two of these were ring-fasciated (4). The specimens were investigated by A. Nessler (17) in 1894. One was 17 mm. long, the cavity being 2 mm. in diameter, the tube spreading into three lobes at top; the other was divided above on one side, and continued as a flat fasciation. Internally the structure agreed exactly with that described in the sweet potato, except that the spiral tracheæ of the internal bundle system were the elements persisting farthest down into the pith. In the sweet potato, the phlœm elements were observed lowest down.

Another stem of the same origin, investigated by Nessler, contained a ring of vascular bundles in the pith which united with the normal ring above but vanished below as in the other cases. The only external sign of this was an increase in the diameter of the stem. Was this a case of arrested ring-fasciation?

The two doubtful cases of ring-fasciation are: First, a "tubular stem of *Sempervivum*" recorded by Masters (12), and, second, two fasciated sweet-peas with "large tubular stems," reported by C. P. Qualch (19); from inside the hollow of each of these arose a single stem, partly free and partly adherent. Probably all of these were cases of ring-fasciation.

Concerning Michelis' dandelions (14, 15), Nessler is doubtless right in considering them as fusions, and belonging to quite another category. Neither can we get much light on the present subject from normal structures, as the fruits of *Ficus* or the "hips" of roses, for in these the morphological apex of growth lies in the bottom of the cup-like body. The occurrence of two of the three true ring-fasciations in fasciated races, namely, *Veronica longifolia* and sweet potato, and the frequency of ring-fasciation and abundance of plain fasciation in the latter plant, make it highly probable that the two phenomena are but phases of one and the same condition. The very frequent splitting of the tubes into one to three or four bands makes the correctness of this view almost certain. An injury to the growing tip has been suggested as a cause of ring-fasciation, but no sign of such has been found in any of the cases examined. We may therefore consider that whereas plain fasciation occurs when the meristem is so stimulated (by overfeeding or otherwise) as to cause it to spread out in two opposite directions and become linear at the apex, ring-fasciation occurs when the same stimuli, operating for a time in radial symmetry, cause a spreading of the meristem in all directions equally, giving rise to a circular apical region.[1]

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[1] Since going to press two ring-fasciated shoots have appeared on a plant of *Symphytum caucasicum*, growing in the University Botanic Garden.

EXPLANATION OF PLATE XIX.

Fig. 1. Internal bundle system of a small ring-fasciation. Photomicrograph, magnified about 60 diam.

Fig. 2. External bundle system of ring-fasciated stem, being altogether normal in structure. Photomicrograph, magnified as Fig. 1.

Fig. 3. Remnant of vascular bundle in the midst of the pith at the basal end of the ring-fasciated region.

Fig. 4. Diagram of Ring-fasciated Stem.

Ep. = epidermis.
Ph. = phloem.
Co. = cortex.
Ca. = cambium.
B. S. = bundle sheath.
Xy. = xylem.
B. F. = bast fibre ring.
I. Ph. = internal phloem.
Med. = pith.

Capital letters indicate external (normal) bundle system; small letters indicate internal (anomalous) system.

Fig. 5. Portion (taken about 12 inches from the apex) of a very large ring-fasciation. The ring splits above and spreads out, forming a plain fasciation. Magnified one and one-half times.

Fig. 6. Apex of a complete ring-fasciation, showing the internal cavity and the crowded leaf rudiments. Natural size. From sketch by Miss M. Mackenzie.

LITERATURE.

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The Beach Plum, Viewed from Botanical and Economic Aspects.

(*WITH PLATES XX, XXI.*)

By Professor J. M. Macfarlane.

The publication of Darwin's "Animals and Plants Under Domestication" made us familiar with a large body of facts bearing on the variations of plants under man's selective influence. It has been frequently objected, that the numerous cases of variability there quoted, are of little value as guides to the interpretation of natural phenomena, since they were produced under the guiding agency of man, and could not be paralleled in nature. Recent studies have in part removed such objection, but every contribution to the subject of variation in the wild state has a special value. The subjoined observations are brought together from study of a wild plant that shows marked variability along several lines.

It has been frequently accepted—and for good reasons—that marked variations are primarily due to the combined action of such environmental agents as light, heat, moisture, soil surroundings, or winds acting to different degrees in different localities. The writer gathered plants of *Salvia lyrata*, some years ago, south of Savannah, Ga., that were so vigorous in growth, so abundantly bloomed, so large and richly colored in the corolla and even leaves, as to suggest its being a different species from our Northern type. Careful study showed it to be an improved variety of the species, called forth probably by the warmer, brighter climate of that region.

But when plants that are specifically identical exhibit marked variation in the same geographic and climatic centers, and where accordingly they are all exposed to apparently like environmental conditions, the case becomes one of exceptional interest and perplexing explanation. Such a case is that now before us.

The Beach Plum (*Prunus maritima*) is abundant along the coast regions of the Eastern States from Virginia to New Brunswick. During the past eight years the writer has studied it at Cape May Point, Ocean View, Island Heights, Martha's Vineyard and near Falmouth, Mass. In these localities it may cover areas from ten to two hundred acres in extent, often to the exclusion of other plants. Considerable information has also been obtained for me by Mr. H. S. Conard and Mr. George Wilson, from Wildwood, Cape Cod and Plymouth, at all of which places it is abundant. It is usually met with, and seems most prolific, in expanses of soft often drifting sand in sight of the sea, or even along the shore, where it may at times be washed by ocean water. Downing says[1] it is "found mostly on the sandy seashore, from Massachusetts to Virginia, and seldom ripening well elsewhere." In New Jersey it may be got sparingly at considerable distances from the sea. Between Manumuskin and Maurice River, about eight miles from sea water, Mr. Le Boutillier has drawn my attention to a number of bushes, some of them old and of large size. At May's Landing, about fifteen miles from the sea, it is not uncommon, and flowers profusely. East of Atco, N. J., about forty-two miles from the sea, are a few apparently wild bushes that look healthy, and are said to fruit well. In all of the above localities the soil is a light loose sand, that makes walking heavy and tiresome.

How the plants reached the two or three last-named localities it is impossible to say. Possibly they represent plant islands left as the ocean has retreated. Possibly the fruit stones were scattered by Indians, who may have brought the

[1] Encyclopædia, Vol. II, p. 889.

fruits from the ocean front. The numerous remains of Indian camps at Maurice River might suggest the latter, which would be strengthened by the knowledge we have of extensive Indian migrations.

Fully six years ago, near Island Heights Junction, my attention was attracted by the extensive areas covered by the plant. Just before leaving the locality in the middle of August the fruits were becoming ripe. The extreme variations shown in size, shape, color, taste, consistence, and maturation period, suggested the desirability for further observation. Four years ago, at Martha's Vineyard and Falmouth, additional facts were obtained, but the most careful and extensive studies were made during last and the preceding summers at Cape May Point. Immediately south of the last named locality are hundreds of acres of loose sandy soil adjoining the shore, and covered with continuous growths of the plum, or this interspersed with gnarled and weird-looking specimens of the Red Cedar. The earlier varieties begin to ripen there from August 10 to 20, and a continuous supply can be had till September 10. Thereafter a few good varieties may continue to ripen till September 20, but after that date an abundance can be had only of a hard, greenish-purple variety of medium to small size, that sweetens imperfectly late in October.

The Beach Plum is a bush that ordinarily grows to a height of five feet to six feet, and may then be from ten to twenty or twenty-five years old. Plants from ten feet to twelve feet high are occasional, and indicate an age of fifty to sixty years. These latter as a rule bear fruit as abundantly as do the younger specimens. As shown by Plate XX the fruits mature in close-set clusters on the older wood, each cluster being made up of several minor clusters of two to five. These give to the bushes in autumn an extremely rich aspect.

My studies as yet have been mainly confined to fruit and stone variations, and these alone will be treated in the present paper. But noteworthy variations occur in the plants, as regard size and vigor, color of the twigs and buds, also size, shape and hairiness of the leaves. Such variations have already attracted attention. Thus Gray says "It varies when at some distance from the coast (New Jersey and southward), with the leaves smoother and thinner, and the fruit smaller." That it varies is undoubted, that it does so geographically as above stated is not the writer's experience. Wood says:

"The fact is worthy of emphasis that on any one bush fruit and seed are very uniform in every respect, the greatest difference in individual fruits from any one plant being in the size, though even in this the variation is comparatively slight."

The important lines of variation shown by the fruit may be arranged under the following heads: (*a*) color, (*b*) weight, (*c*) size and shape, (*d*) consistence, (*e*) taste, (*f*) time of maturation, (*g*) comparison of the stones.

(*a*) *Fruit-Color.*—The color variations of the fruit constitute the most striking difference to the eye, but it would be impossible to segregate groups that are sharply demarcated from each other. The average color of about 65 per cent of the fruiting plants of any one locality is a rich black-blue, that is slightly lightened in effect by a whitish waxen bloom. This seems to be the color which, by natural selection, becomes dominant in the localities I have visited, though it is not to be regarded as the primitive type. From more primitive forms than this, three diverging lines of variation seem to be traceable, one gradually receding to more and more primitive types, a second advancing to the more highly specialized forms that end in the yellow fruited examples. The former is made up of those types which pass in color from black-blue to bluish, bluish purple, green purple and finally purplish green. The last of these seems, for many reasons, to be the primitive form. Along the second line successive steps can be taken through bluish purple, purplish red, and reddish yellow, till a pure orange or gamboge yellow is reached, which in tint will compare with the finest cultivated plums of the Golden Esperon section. Some of the reddish yellow and yellow types are flecked with faint white specks or blotches, while others are quite uniform. The third line culminates in the finest blue-blacks.

The surface variations thus shown, usually are an index to corresponding variations in color of the pulp. In the blackish blues, this is of a dull reddish purple hue; in the bluish purples of a pale greenish red; in the purplish greens of a light watery green, while in the red-yellow series it passes from shades of pale watery red to watery yellow.

The color variations of the stones follow those of the fruits though to a less marked degree. The stones of black-blue fruits are of a purplish red hue when fresh, changing to dull red when dried. Those of the purplish-red fruits are of a faint red or reddish yellow hue; of the red fruits the stones are faint yellow. Finally, the yellow fruits have clean whitish yellow stones.

(*b*) *Fruit-Weight.* — Under this head it is possible to introduce exact statistics, and in the subjoined table some of these are grouped, as drawn from study of twelve distinct varieties. The bracketing of these under five groups has reference, more or less, to the color relations already discussed, and it will be seen that one of the first group (No. 7) of a bluish black hue, excels in every detail. The types that are poorest in flesh, and have relatively the heaviest stones are those already alluded to, as of a purplish green color, and which suggest the possession of primitive characters in every respect.

Number 12 of the table is a remarkable variety, represented only by three bushes at Cape May Point, that are widely apart from each other. They agreed in being slender bushes, about three and one-half to four feet high, that produced fine twigs clothed with small leaves. The fruits were of small size, had a pale gamboge colored skin, clear pulp, and a small whitish yellow stone. It may fairly be regarded as the most aberrant type encountered.

In estimating weights, twelve plums were selected at random from one type and weighed in mass. The average weight of one was therefrom deduced. Specimens that appeared to be the smallest, medium and largest of this type were then selected and weighed individually. A final estimate was obtained by comparing the mean of these with the result of the former weighing:

		Fruit weight.	Stone weight.	Flesh.
Purple-green to blue and blue-black.	No. 1	1.26 gr.	.43 gr.	.83
	No. 2	1.53 "	.41 "	1.12
	No. 3	1.55 "	.38 "	1.17
	No. 4	1.68 "	.39 "	1.29
	No. 5	2.27 "	.55 "	1.72
	No. 6	2.58 "	.47 "	2.11
	No. 7	3.61 "	.60 "	3.01
Reddish purple.	No. 8	2.93 "	.59 "	2.34
Red.	No. 9	2.24 "	.53 "	1.71
Red-yellow to yellow.	No. 10	3.32 "	.65 "	2.57
	No. 11	2.12 "	.42 "	1.70
Small yellow.	No. 12	1.45 "	.28 "	1.17

From the above table the following approximate estimates can be made, in comparing fruit weight with stone weight:

Stone weight one-sixth that of fruit weight = No. 7.
Stone weight one-fifth that of fruit weight = Nos. 6, 8, 10, 11, 12.
Stone weight one-fourth that of fruit weight = Nos. 3, 4, 5, 9.
Stone weight one-third that of fruit weight = Nos. 1, 2.

The order, in weight, of stones from the lightest to the heaviest is as follows: Nos. 12, 3, 4, 2, 11, 1, 6, 9, 5, 8, 7, 10.

The extreme importance of such marked variation conditions, as affording starting points for successful cultural results, will be emphasized in a later part of this paper. But the statistics are of high scientific interest as proving that within limited centers of plant distribution, types may originate apparently from no traceable environmental causes, which vary powerfully in the very parts—fruit and stone—that contribute to the perpetuation and distribution of the species.

(*c*) *Fruit Size and Shape.*—The two smallest fruits observed, belonged to plants that differed in nearly every other respect, viz.: Nos. 1 and 12 of the above table. Both were spherical fruits that had a cross-measurement of 11 to 13 mm. Occasionally bushes of type 1 may be encountered in which the shape tends more toward the spheroid. Intermediate gradations can be traced from these to the largest, which was spheroidal in shape and measured 21 mm., but the most noteworthy in shape of its fruits was No. 3 of the table. Two bushes were observed, the fruit was oval in outline and measured 12 by 17 mm. The shape and size were surprisingly uniform over each plant, and indicated a fixed hereditary condition in the plant.

As in our varieties of the cultivated plum, so in these a pronounced carpellary sutural groove may occur in some, only a faint trace of it in others, while in Nos. 4 and 10 of the table the groove is obliterated.

(*d*, *e*) *Fruit Taste and Consistence.*—The numerous variations shown, in both of these respects, by the cultivated plums of our day are well known. Similar variations are seen in the wild beach plum. Average fruits have what might be described as a slightly watery pulp, that is, instead of possessing the firm continuous pulp of our best plums, it here consists of pulpy tissue filled in between with softer, almost watery material that inclines to drip. This is true of the finest blue-black fruits of large size, such as No. 7 in the table, and even of the small blue-black types. The purplish-red and yellow fruits are of firmer texture, while No. 1 of the table has a hard, close pulp, that is small in quantity and poor in quality.

A few, like Nos. 2 and 5, have rough cling-stone fruits, the firmer pulpy bands in such specimens being adherent to the stone, some like Nos. 7, 8 and 11 are only slightly cling-stone, while the majority are free-stone. The last condition is specially true for the red-yellow and yellow varieties.

The taste of the fruits is determined largely by the presence or absence of sugar, tannin and acid constituents. Wide variations occur in the relative quantity of these. Thus, in small, greenish-purple varieties like No. 1, that mature late in the season, the amount of sugar is small, the proportion of tannin large, so that they are inedible. Increase in size of the purplish-blue and blue-black types is generally accompanied by marked increase in the sugar content, and reduction in the tannin when the fruit is fully ripe. On this account the taste of No. 7 is highly agreeable, and ranks it with the best of our summer fruits. On the other hand, No. 5, even when ripe, has a decided tannin grip as in some of our coarser bird-cherries. In the purplish-red and red fruits of Nos. 8 and 9, the amounts of tannin and of acid are considerable, but as we pass to the reddish-yellow and yellow varieties, the tannin decreases, and the acid slightly so, till in Nos. 10 and 11 of the table the fruits become of superior quality. It will thus be observed that the coarsest types of fruits are the small, late maturing ones of greenish-purple hue, and that the blue-black, at the end of one series of types, also the red-yellow and yellow at the end of another, are almost free from tannin and are rich in sugars and mild acids.

(*f*) *Maturation in Fruit.*—In many of our highly-cultivated fruits, such as the apple, pear, peach and plum, the period for ripening of the different varieties may extend over two to three months, but we rarely see such variation on a wide scale in a wild type. The beach plum presents such a condition.

In an average season along the New Jersey coast, the earliest bushes to ripen are those which bear reddish-purple fruits. These mature from about August 10 on to the first week in September. Meanwhile the blue and blue-black, also the yellow varieties are coming forward, and give a succession of fruits from about August 18 to September 10. These again are followed by the small purplish-blue types, which ripen from about September 1 to 20. Finally, during the latter part of September and on to October 15, the medium-sized and small greenish-purple varieties ripen. These last seldom seem to attain the consistency—even in October when they fall—that we call ripeness. They are then decidedly firm in the flesh, and abound in tannin and acid constituents.

(*g*) *Variations in Fruit Stones.*—Darwin has drawn attention to, and has figured, varieties of the fruit stone of the cultivated plum.[1] Though nothing has been observed in the beach plum that would compare with these, the differences are nevertheless suggestive. We may at once compare two rather extreme cases presented under Nos. 7 and 12 of the table. The former are oval, compressed in shape, are slightly rough and have a well-marked ridge that traverses one side and ends in a slight beak. The color is a dull crimson-red, the weight is .60 gram, and in relation to the pulp the stone is of cling-stone variety. The stones of No. 12 are of nearly spherical shape, smooth and have only a faint indication of a ridge The color is a clear liquid yellow, the weight is .28 gm. and the pulp is entirely free from the stone.

A COMPARISON OF THE BEACH PLUM WITH THE CULTIVATED PLUM AND ECONOMIC SUGGESTIONS ARISING THEREFROM.

It seemed advisable during the progress of the present inquiry to institute a comparison between fruits of the beach plum and those of the garden plum. Authorities differ as to the wild parentage of the latter, but most agree that *Prunus*

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[1] "Variation of Animals and Plants," Vol. I, p. 366.

insititia or *P. domestica* started our present varieties. I have failed to learn what degree of variability, if any, either of the above species shows in nature, but Dr. Erwin F. Smith informs me that the sand plum of the Northern States varies as does the beach plum.

It is to be regretted that in the works of Downing and others no exact estimate is given as to fruit and stone weight. The following have been gathered by the writer: Several weighings of the Dawson plum—a variety of damson—gave for fruit weight 4.30 gms., for stone weight .70 gm., so that the ratio here of stone to pulp is about one-sixth, or the same ratio in a cultivated fruit, as already exists in the finer varieties of the beach plum. The "California Tragedy," a reddish-purple fruit, weighed 31 gms., while the stone weighed 1.74, or in ratio as one to eighteen. A market greengage weighed 35 gms. and the stone 2.42 gms., or in ratio as one to fourteen and seven-eighths. A specially fine greengage bought in market, and which seemed to answer to Lawrence's gage, weighed 65 gms., the stone 2.25 gms., or in ratio as one to thirty.

One is immediately impressed by the wide differences shown between pulp and stone in the commoner and finer varieties of cultivated plum. But this is exactly what cultivation has been proved to accomplish for our best fruits. Darwin gives a very pertinent illustration in the English gooseberry. The wild fruit weighs about 120 grains; in 1786 samples were on exhibition which weighed 240 grains; in 1830 the weight was 781 grains, and in 1852 the limit of 896 grains was reached. This is fully seven times the weight of the natural fruit.

If we suppose the wild ancestor of the garden plum to have weighed about three grams (since the weight of the cultivated Damson already given was 3.30) this would indicate that cultivation has increased it fully twentyfold. But the weight of average beach plums is 2.50 grams, while fine varieties, such as No. 7, average about 3.50 grams. It seems not unreasonable to suppose, therefore, that excellent starting points for future efforts in cultivation already exist among native strains.

Features specially commending it for cultivation are its constant growth amid loose, open sand, and in proximity to or in immediate contact with the sea. As shown in Plates XX and XXI, it fruits heavily, while the quality of the finer varieties excels that of any other native fruit, in the writer's estimation.

Along the sea front, from New Jersey to Massachusetts, many thousands of quarts are gathered annually, which are used in part as a delicious table fruit, but in larger part are converted into jelly preserves. Through the kindness of friends I have learned that a considerable trade in the fruit exists along the Cape Cod and Plymouth coasts, where it is sold at from five to ten cents a quart, so that already it is a commercial article.

By judicious cultivation and selection it is certain that many and finer varieties might be secured, since the plant in the wild state has already developed so favorably. The species seems to have been selected in nature as a type of fruit-plant that is specially adapted to its surroundings. The environmental areas covered by it include hundreds of thousands of acres of our eastern shore land, where no other fruit plant naturally grows, if we except in places the sand dewberry. Its roots often travel far and act as good sand binders, as well as extensively ramified absorbents of soil food.

Against these commendable qualities are to be reckoned two objectionable features which might, for some varieties only, militate against their economic value. One of these is the soft, pulpy consistence of the fruits in such types as Nos. 5–7 of the preceding table. Thus when the fruit is plucked from the stalk, considerable "bleeding" occurs, if these are heaped on a dish or in a basket for a few hours. If, however, the fruit and stalk be both plucked, these have a finer and more natural aspect than has the fruit alone, while bleeding is thus completely prevented.

The second and more serious objection is the frequent puncture of the young fruit—probably by some insect—and the resulting formation of a hard, black core of material running through the pulp into the stone. Such a condition, however, is only shown by certain types of blue-black and blue-purple color, and is almost, or entirely, absent from others, notably from the red-yellow and yellow varieties.

Where such puncture cores are developed, they secrete or attract around themselves tannin products, that give a harsh, disagreeable taste to the pulp. It has been impossible as yet to determine the animal that forms them. The effects resemble those caused by the plum weevil, though no larva has as yet been noticed in the fruit. When a variety of the Beach Plum is liable to this foe, it is difficult to find one sound and sweet fruit on any bush, on the other hand many, and some of the best varieties are free from it in every plant.

It is highly desirable that tests be made of the plants on prepared sandy land where it might be manured, pruned, irrigated if necessary, and the results accurately recorded. We would venture to entertain the hope that this hitherto wild plant might become, under fair cultivation and judicious selection, one of our most valuable fruits, and one which, for about two months of every year, might supply our markets with a cheap and delicious fruit.

THE RELATION AND POSSIBLE ORIGIN OF VARIETIES OF THE BEACH PLUM.

When so many varietal forms are shown in fruits of a single species, it might be supposed that such variations would originate only in one or a few centers that were recently connected geographically with each other. Instead of this most of the types already described can be found in close proximity, at all of the localities already named, from Cape May Point in the South to Falmouth and Martha's Vineyard in the North. This might lead us to suppose that in all these centers agencies were at work, that so acted on some primitive type or types, as to cause such to branch out into the varieties described.

Before discussing this further, it may be well to adduce some evidence in favor of the ground already taken that No. I of our table or some closely related form represents a primitive ancestral type that has come down to us, and has been the starting point, in all probability in the past, of most if not all of the varieties described. In nearly all the natural orders of plants that contain succulent fruited genera, the species that have the largest and richest fruit pulp are the most highly evolved, since seed dispersion by animals is increasingly aided by increased succulence. We can likewise accept it as proven that the primitive fruits were dry and that increasing succulence indicates advancing specialization. But as with flowers, so with fruits. The most primitive are of a green or semi-green color. Type I would conform to such a requirement better than any of the others. The large amount of tannin in some varieties, particularly in specimen I, though it might prove to be protective against certain hurtful agents, seems rather to indicate a primitive condition, derived from a dry fruited type in which tannin is a frequent constituent. The practical disappearance of it from the finer fruits of the Beach Plum, and the abundance in them of sugar, favor the view that the varieties most rich in tannin are the most primitive forms.

As regards the lateness of ripening of No I, and a few related examples, this does not seem to be a matter of special importance. It is well known that several late-maturing apples and pears rank among the choicest, sufficient proof this that mere lateness of ripening need not indicate a poor or primitive fruit. The small amount of the fruit pulp and the small size of the seeds in specimen I, are additional proofs that this is a primitive form, rather than a degraded one.

Until better and more conclusive evidence is obtained, we may accept the above as probable, and now try to determine the cause or causes for the origin of the varieties. It deserves to be emphasized, that most of our cultivated plums, and also the sand cherry as well as the beach plum, show fruit and seed variations exactly along the lines of those now described. This might indicate that all of these are comparatively recent evolutions from a common stock that acquired these varietal variations and then branched off into specific lines of development in stem, leaf and flower, without losing the fruit and seed variations already acquired in common. From a primitive small, hard, semi-succulent, purplish green, late-maturing type like No. I, we might have in each of the specific groups lines of variation passing from it to greenish purple, to purple, purplish blue and blue black. Or again from the same starting point we might obtain, by predominence of acid cell contents, greenish red, red, reddish-yellow, to yellow.

As to the possible cause for the origin of these varieties, the writer has nothing of value to suggest. Some suggestions may, however, be offered. Thus when study of all the environmental conditions is made, it is seen that these plants usually grow where alterations in the relations of sea and land are frequent. The varieties of sub-soil also vary considerably, and may affect the plants, according to the heaviness or porosity of it. The relative amount of the halogen compounds in the soil round a plant or plant group, may likewise have a determining effect. Exposure to a bright, hot sun, or protection in comparative shade may also have been a determining factor. A fruitful field for observation and experiment is open here as in many other fields, and suggests to us the high scientific value of experimental stations for the study of plant life. Experiments are now in progress that may aid somewhat the solution of these problems.

EXPLANATION OF PLATES XX, XXI.

Plate XX. Branch of Beach Plum, belonging to form No. 6. Reduced one-fourth.

Plate XXI. Branch of Beach Plum, belonging to form No. 4. Reduced one-half.

A Study of the Fertile Hybrids Produced by Crossing Teosinté and Maize.

(*WITH PLATE XXII.*)

By John W. Harshberger, Ph. D.

This study is presented as an appendix to the monograph on maize, which appeared in "Contributions from the Botanical Laboratory, University of Pennsylvania" (Vol. I, No. 2, p. 75).

Professor A. Dugés sent in 1888 to the Cambridge Botanical Garden several maize plants which he had collected at Novo Leon, Mexico. Seeds from these plants were sown at that garden, and the plants which resulted were studied by the late Sereno Watson.[1] Small ears and kernels of the second generation were procured from Cambridge, Mass., and planted in Philadelphia. The flowers and fruits obtained from the plants thus grown were described and figured in the monograph mentioned above. Later, inquiry was made of Dr. Dugés concerning his discovery, and the following letter in French, dated September 22, 1895, and mailed from Guanajuato was received:

"The maize, which Dr. Sereno Watson named *Zea canina* from the examples which I sent him, is known in Mexico as 'maiz de coyote' (*Lupus latrans*), 'teosinté,' 'asesé' or 'café de Tabasco.' We consider it to be *Euchlæna luxurians*, *Euchlæna mexicana* or *Reana luxurians*. It appears that it has been cultivated in Europe and also in Mexico, where it has been grown by Professor José C. Segura, director of the School of Agriculture, City of Mexico. This botanist discovered at the end of three years of careful cultivation in good soil, that it changed to *Zea mays*, and if abandoned to itself under adverse conditions it reverted to *Zea canina*, that is, the same plant had a wild and a cultivated state."

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[1] Watson, Proceedings American Academy of Arts and Sciences, xxvi, p. 158.

Some time elapsed after the receipt of the above letter, when one was addressed to Professor Segura, who replied in Spanish under date of July 2, 1896:

"In reply to your letter the manifest which I sent you with the bags of the seeds explained, that one bag contained seeds of asesé (*Euchlæna mexicana*), another, seeds obtained by hybridizing *Euchlæna mexicana* with common maize. In consequence, that which you term *Zea canina* Watson is not distinct, but a hybrid of asesé and maize. To obtain this product, which is known in Guanajuato as 'maiz de coyote,' you must sow three grains of *Euchlæna*, and at a distance to prevent crowding of the plants, three kernels of maize. As a result of this sowing in the month of July, the teosinté commences to form its floral peduncles, which should be cut off immediately after it appears [emasculation]. In August when Indian corn flowers, the teosinté is pollinated with pollen of corn.[1] The resulting kernels do not show any modification of their form. The succeeding year upon sowing these kernels, a plant of early habit will result showing in the small sized ears, qualities produced by the blending of common maize and teosinté."

Later, on visiting Mexico in the summer of 1896, the writer procured plants and ears of *Euchlæna* and the hybrids, and Professor Segura very kindly gave him permission to publish the results of the inquiry, using in addition the information which he was so generous as to put at the writer's disposal. It might be stated, before passing to a consideration of the results obtained, that the plants which Sereno Watson described as *Zea canina*, and which were afterward carefully studied by the writer, are identical with the hybrids which Professor Segura obtained at the Agricultural School of Mexico.

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[1] It should be noticed here that the two plants are monœcious and protandrous; the order of flowering at Mexico is as follows according to Professor Segura: male flowers of teosinté, then female flowers of that plant and male flowers of corn produced synchronously, then female flowers of maize, so that the physiological adjustments of both plants preclude the use of pollen of *Euchlæna* in the fertilization of maize.

Teosinté, *Euchlæna mexicana*, Schrad., is a plant of several varieties native to Mexico, where the writer found it growing in the Barranca Chica, near Guadalajara. It is grown as a fodder plant in most warm countries, seldom flowering when planted in Europe. The two-ranked ears are clustered in the axils of the leaves, and have one fertile and one rudimentary flower placed in a hardened cup-shaped depression of the rhachis, the lower coriaceous glume closing the mouth of the hood formed by the rhachis (Plate XXII, Fig. 1a.). The male spike, terminally borne, consists of two flowered spikelets, with three stamens in each flower. The female spikelet has two flowers, one perfect, and one anterior and abortive flower. When maize is crossed with teosinté by the use of maize pollen, the hybrid progeny of the first generation is intermediate and shows a much shortened branch in the axil of a leaf with three or four ears clustered together, and surrounded on the outside by leaves, which are usually termed husks (Fig. 2). These hybrid ears resemble in a number of particulars those of teosinté in that they are two ranked with the kernels in the hardened depression of an enlarged zigzag rhachis, which shows the beginning of a cob-like axis, on which in this case the grains are disposed in a distichous manner (Fig. 1). The kernels of this hybrid generation are larger, sharp-pointed and protrude between the scales (glumes) from the cup-shaped depression of the axis, which is in this case shallower than in teosinté. The outer glume, which is hard in teosinté, becomes larger and softer in the hybrid progeny. The axis is still firm, glossy and chitinous. In the second year maize pollen is again used, to cross with the hybrid plants of the first generation. The result of this cross is a form of ear in which the kernels are larger, more rounded and more floury, while the corneous cucullate depression of the rhachis has become smaller and more saucer-like (Fig. 3). The kernels of this generation are usually arranged in a distichous manner. The third year, pollen of Indian corn is again used, and the resulting ears are found to differ by the increase of the number of rows of grains, four or more being found; the pithy axis (cob) now becomes demarcated, and is seen when the ear is broken across (Figs. 4 and 5). The plants of this generation and the fourth (Figs. 6, 7 and 8) are identical with those described by Professor Watson and myself.[1]

The cross thus established between teosinté and maize may be represented as follows:

Euchlæna mexicana Schrad. ♀ × *Zea mays* L. ♂ = *Zea canina* Watson, the "maiz de coyote," or "maiz de los gentiles" of the Mexicans.

Before closing, it is important to refer to two interesting facts. The hybrids described above are known widely throughout Mexico. Dr. Carl Lumholz found ears identical with those of the hybrids among the timid Tarahumara Indians of northern central Mexico, who, in their mountain fastnesses, come little in contact with white men. Dr. Nicolas Leon also informs me that the hybrids are grown by the Mixes and Zapotecs inhabiting the State of Oaxaca, and that there the plant is quite common.

Corn smut (*Ustilago zeæ*) also occurs on the hybrids especially those of the fourth generation, as so clearly shown in the photograph (Fig. 9).

CONCLUSIONS.

With these interesting hybrids before him, one is tempted to theorize. Is *Zea mays* a true species, or is it a cultivated race or variety of *Euchlæna mexicana?* If the latter, then *Zea canina* becomes a mongrel plant. Or is Indian corn a species of *Euchlæna*, closely related by consanguinity to *Euchlæna mexicana?* These questions can only be answered by the discovery of the wild plants concerned in the problem of the origin of maize.[1] The following formulæ present the hypothetical position of the writer upon this question. Common names are used to prevent the confusion of synonyms.

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[1] A very fair but reduced illustration of the ears of the fourth hybrid generation is to be found in my monograph.—Bot. Contrib., Univ. Penn., I, plate xv, fig. 9.

Teosinté ♀ wild, crossed by the partially ameliorated progenitor of Maize (a species of *Euchlæna*) ♂ probably produces the ordinary Indian Corn (*Zea mays* L.), and the second cross known by actual experiment to occur, viz:

Euchlæna mexicana Schrad. ♀ × *Zea mays* L. ♂ = *Zea canina* Watson, the "maiz de coyote" of the Mexicans.

Until this purely theoretical view is established, it is the best and the safest course to pursue to keep closely to the known facts concerning these cereal plants, teosinté and maize, relegating theoretical considerations to the place kept for unestablished hypotheses, the botanical Gehenna.

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[1] 1896. Harshberger, Garden and Forest, IX, p. 522, where theoretical considerations are given.

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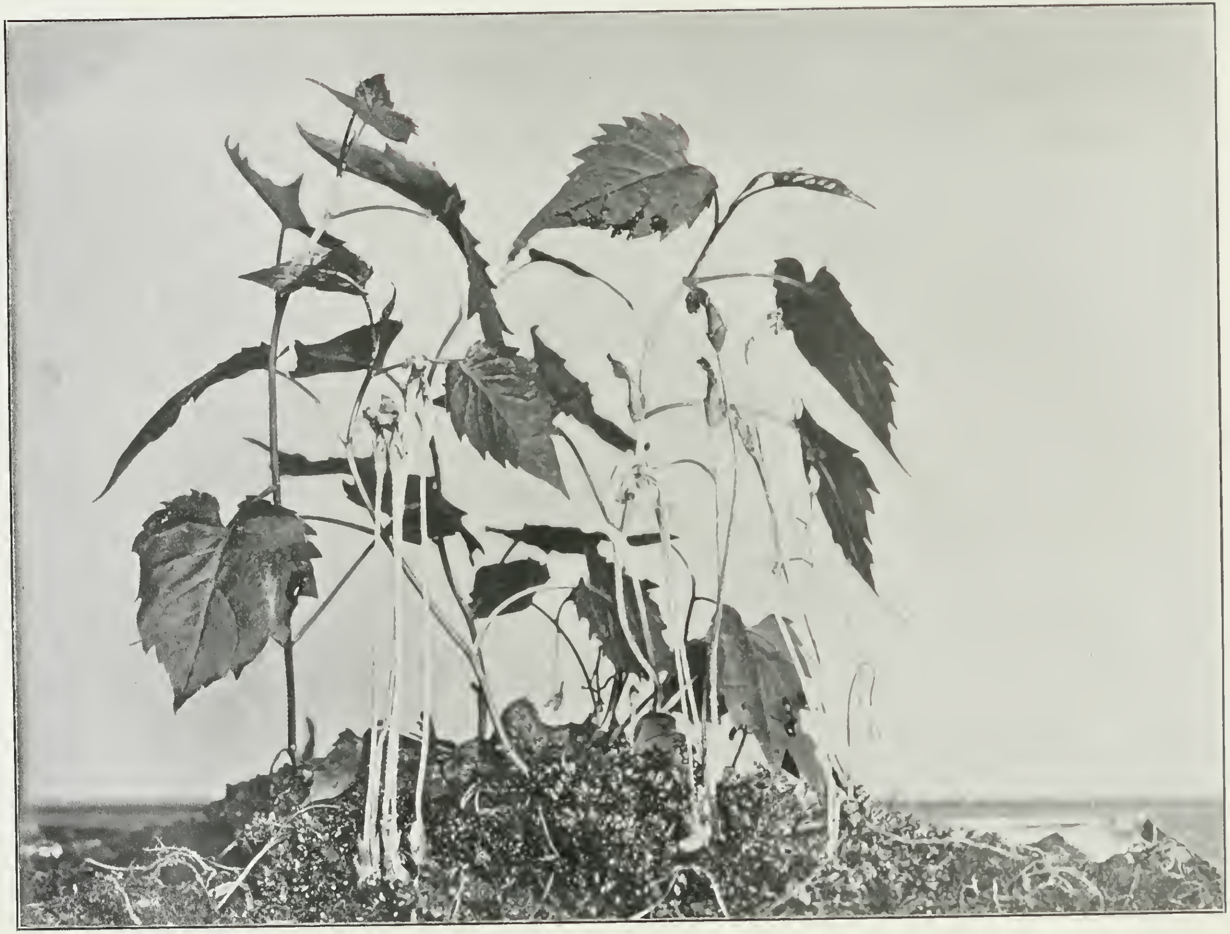

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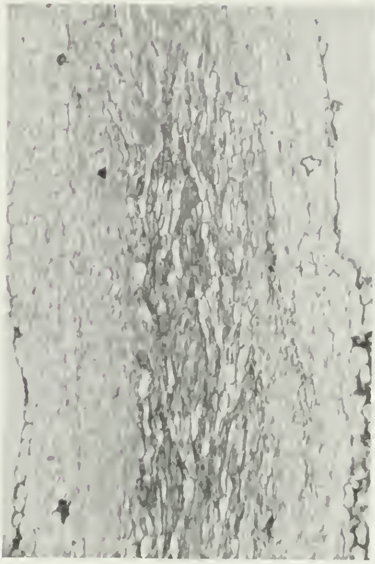

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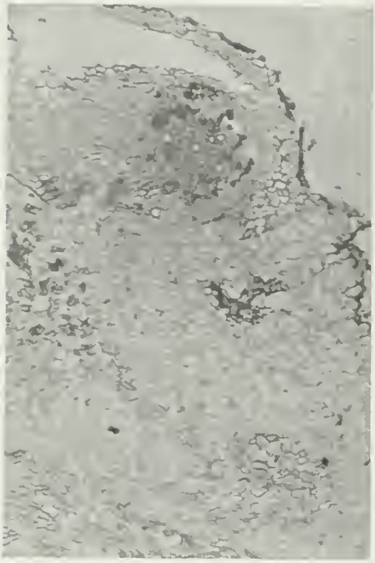

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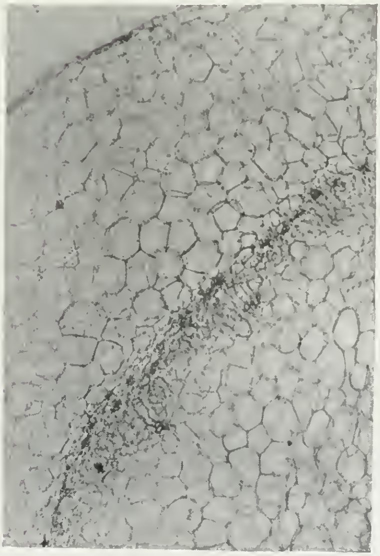

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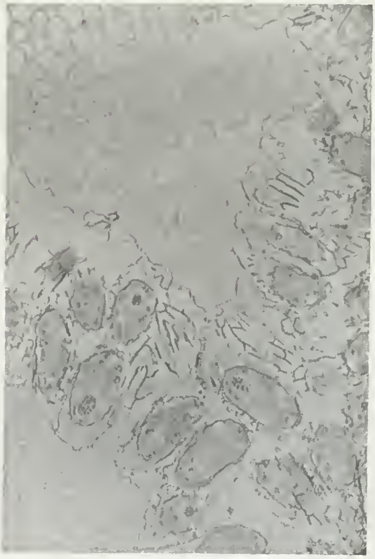

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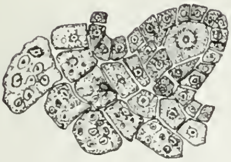

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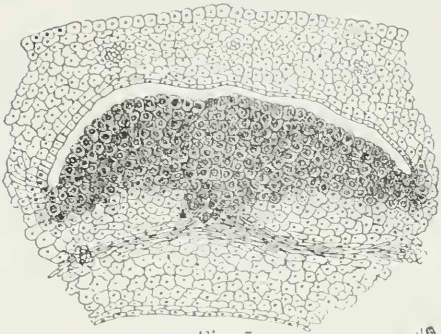

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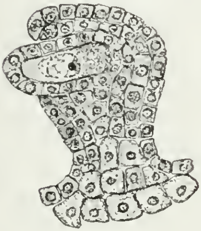

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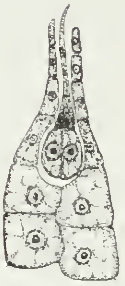

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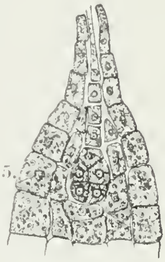

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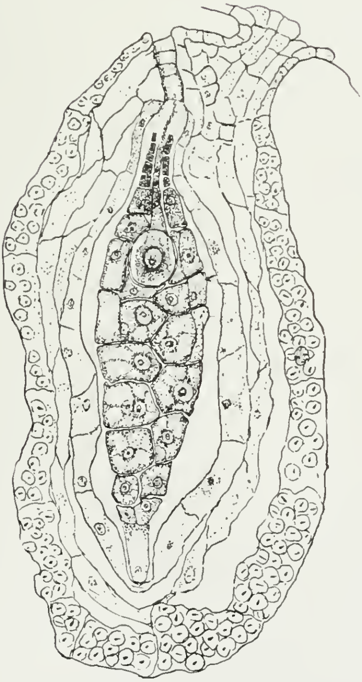

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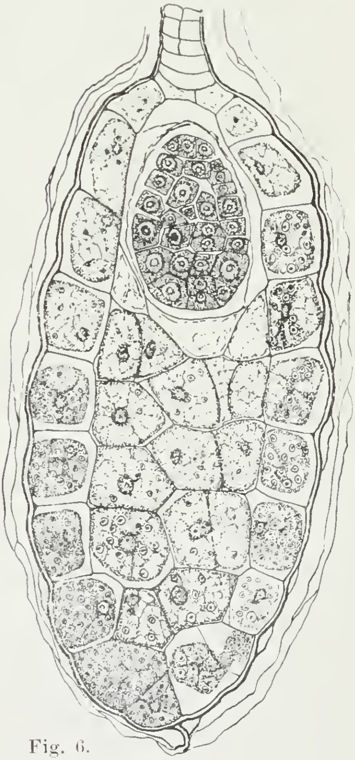

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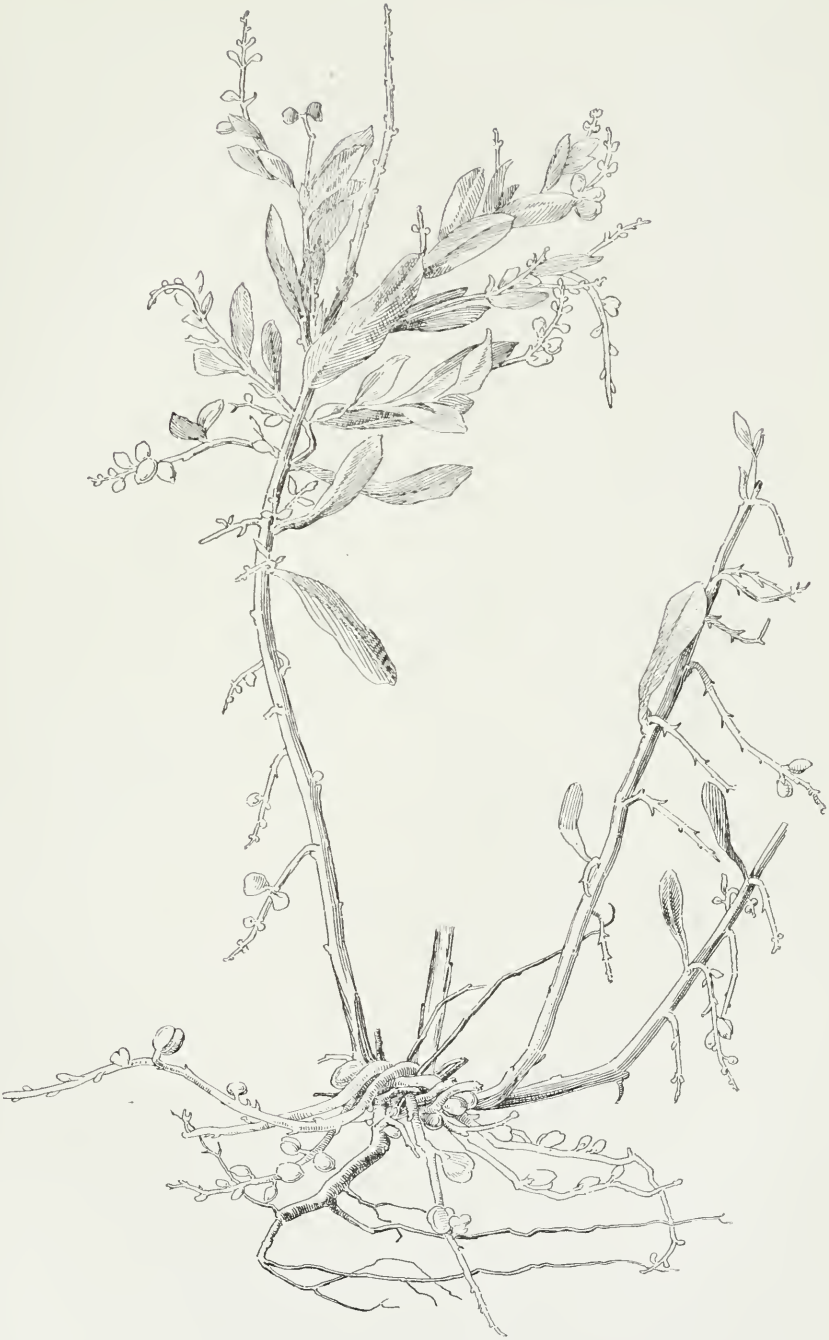

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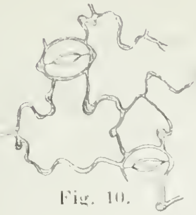

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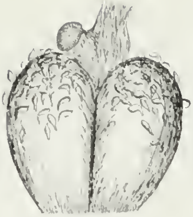

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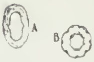

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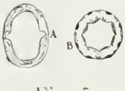

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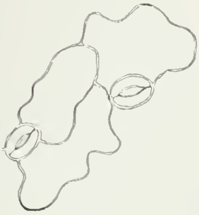

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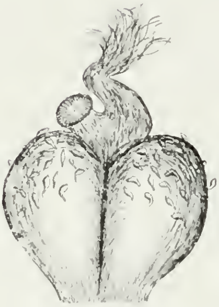

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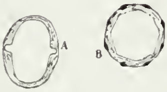

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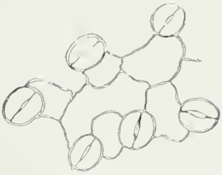

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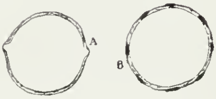

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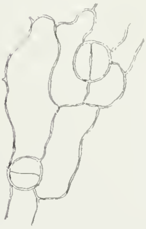

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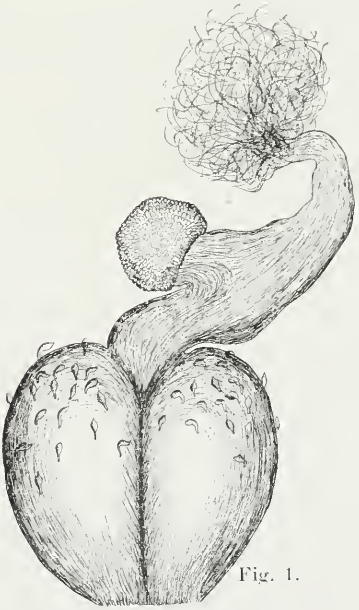

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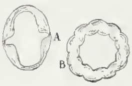

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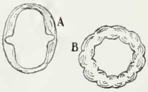
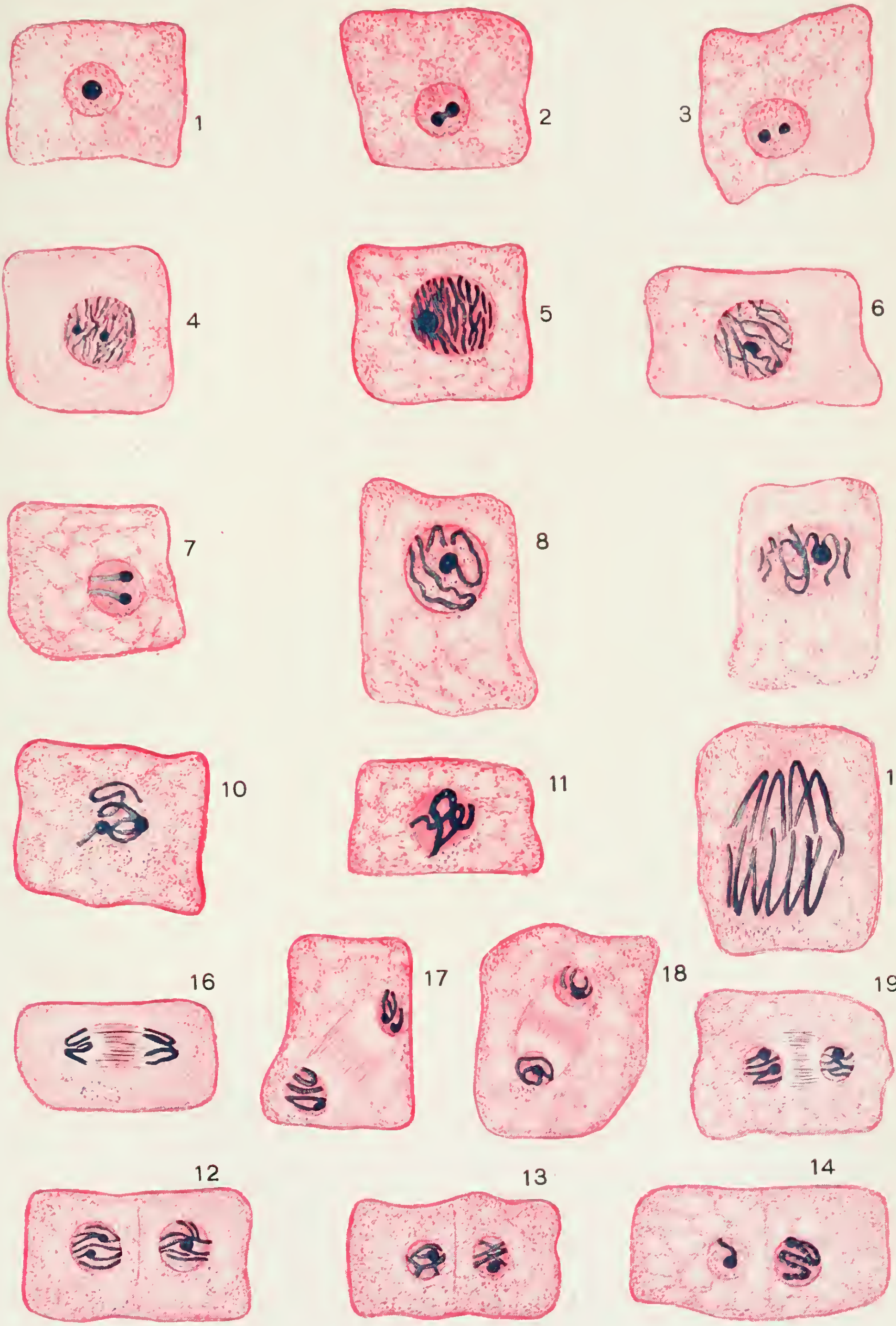


Fig. 8.

SHAW ON POLYGALA.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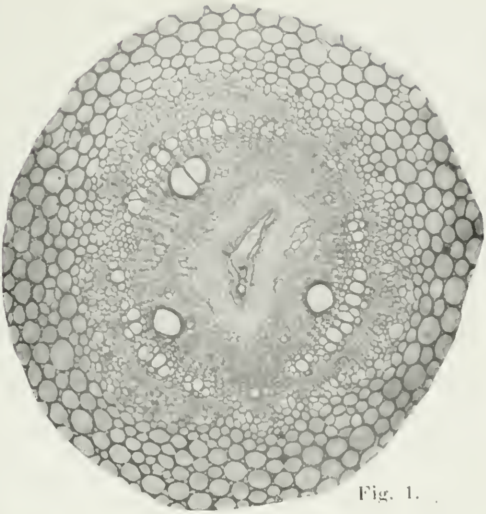
GARDNER ON CELL-DIVISION.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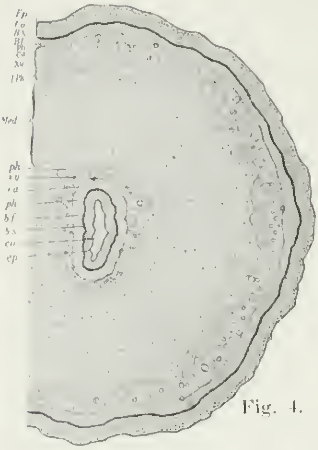
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Fig. 4.

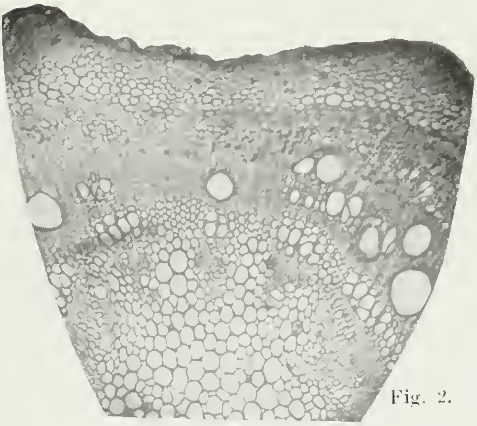


Fig. 2.

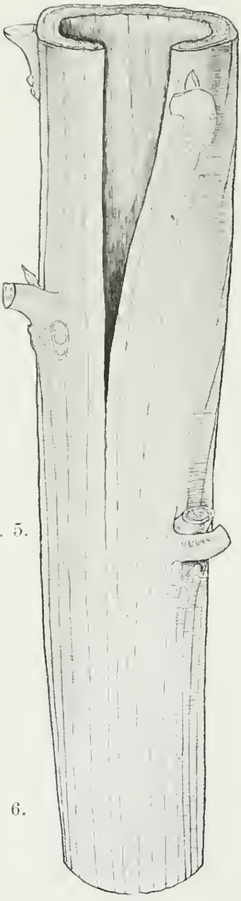


Fig. 5.

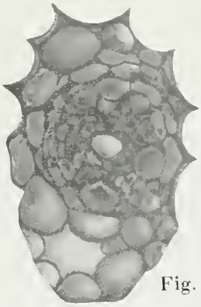


Fig. 3.

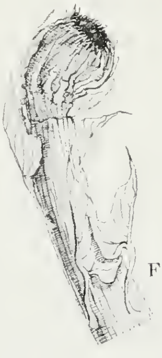
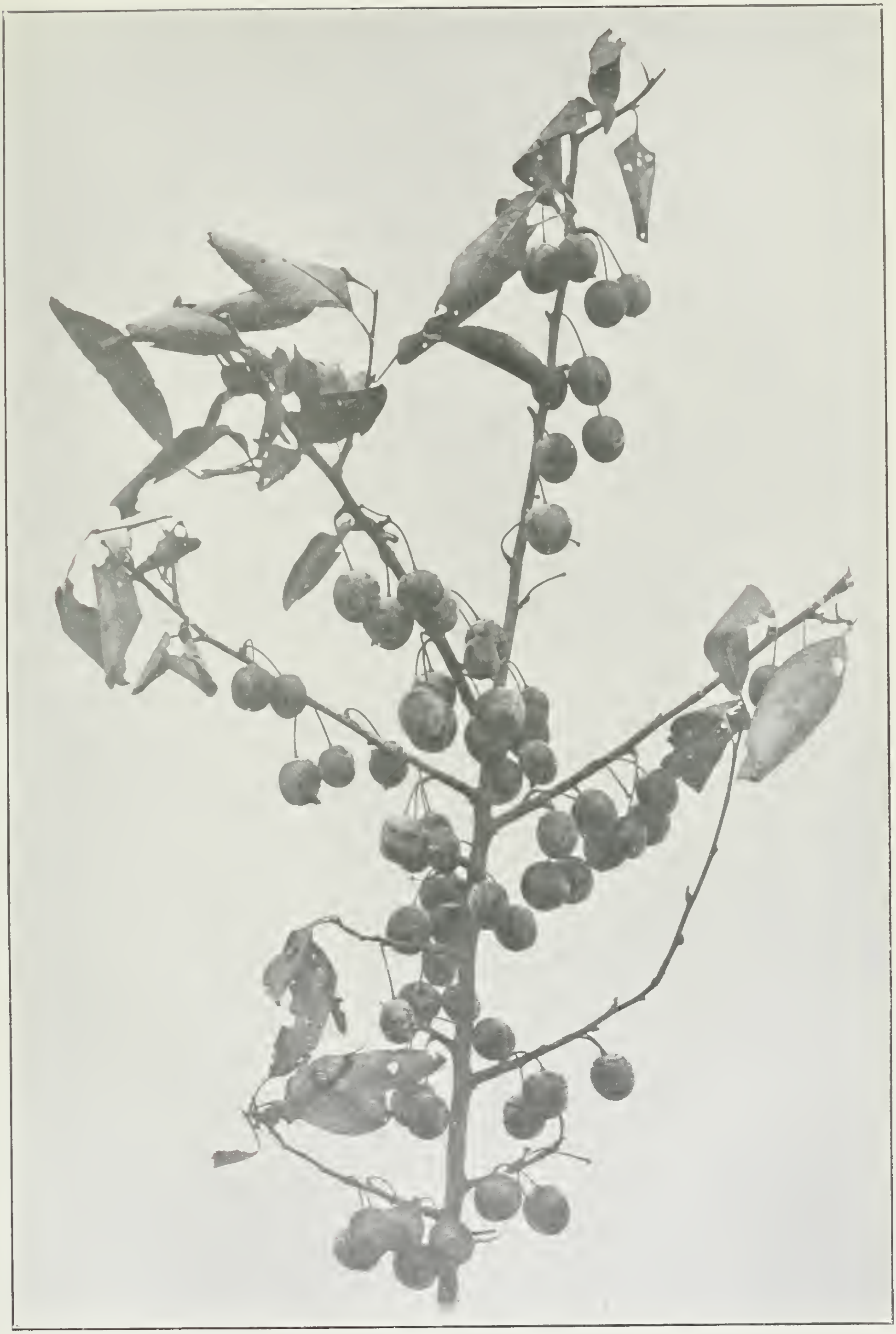


Fig. 6.

Conard on Ring-fasciation.

MACFARLANE

Beach Plum.



Macfarlane on Beach Plum.

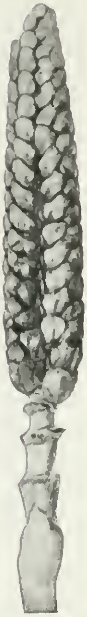


Fig. 6.

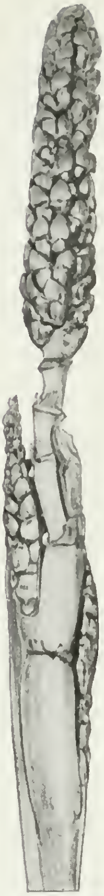


Fig. 8.

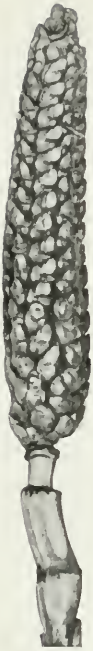


Fig. 7.

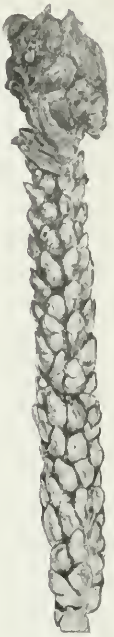


Fig. 9.

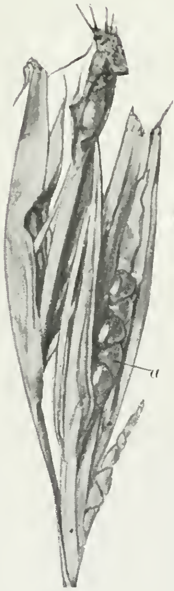
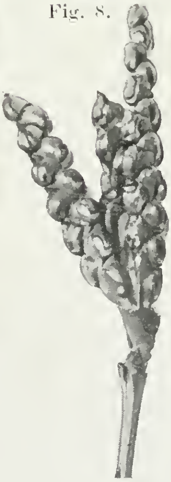


Fig. 1.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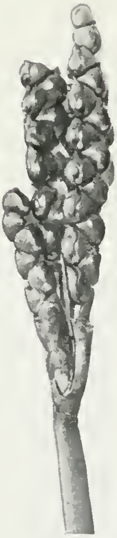
Fig. 3.

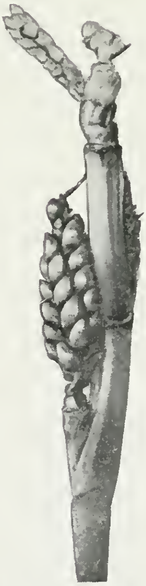
Fig. 4.

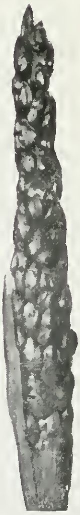
Fig. 5.

Harshberger on Maize Hybrids.

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UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
1904

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Botanical Laboratory of the University of Pennsylvania

VOL. II, NO. 3.

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A Comparative Study of the Cytology and Movements of the Cyanophyceæ.

(*WITH PLATES XXIII-XXV.*)

Thesis presented to the Department of Philosophy, University of Pennsylvania, May 1903, in partial fulfilment of requirements for the Degree of Doctor of Philosophy.

Read before the Botanical Society of Pennsylvania, October 18th, 1902, and May 15th, 1903.*

BY ORVILLE P. PHILLIPS, M. S., PH. D.

The problem of heredity has been attacked from many sides. Various theories concerning the transmission of characteristics, whether acquired or otherwise, have been given to the world, and as many have been withdrawn or proven untenable, at least by some authorities. From Thales to

---

* In view of the recent appearance of three valuable contributions to the above subject, it may be stated that Dr. Phillips' completed thesis was placed in my hands on May 3d, 1903, and has been in my care since. Its publication has been unavoidably delayed till now, but no alterations have been made on it. While the views here set forth can claim no priority over those already published, their appearance may aid in the settlement of disputed points.—J. M. MACFARLANE, Editor.

Darwin the efforts of biological investigation have been largely directed towards the solution of this problem. But many of the investigators worked wholly upon gross anatomy, as it might be termed, in distinction to the study of the cell. They failed to realize that, as the alchemist was being driven from masses to atoms as a basis for his investigations, they too, if they were to solve the questions which interested them most, must get down to the minutest structural elements of plants and animals.

The introduction of the cell theory by Schleiden in 1838 completely revolutionized the older ideas of transmission, and concentrated attention upon the cell and its contents as the true vehicles of heredity. Even here again war waged between conflicting theories. Darwin's "gemmules," Weismann's "germ plasm," His's "germinal location," Naegeli's "idioplasm" have all had their turn in the arena of conflict, and from this discussion has come the conviction that in the chromatin and linin of the nucleus is to be found the real hereditary material as is well indicated by Wilson (86), who says: "In accepting this view we admit that the cytoplasm of the egg is, in a measure, the substratum of inheritance, but it is only by virtue of its relation to the nucleus, which is, so to speak, the ultimate court of appeal. The nucleus cannot operate without a cytoplasmic field in which its peculiar powers may come into play; but this field is created and moulded by itself. Both are necessary to *development;* the nucleus alone suffices for *inheritance* of specific possibilities of development."

But the cells in which nuclei have been undoubtedly demonstrated in the past are in the plants above the Cyanophyceæ. In the group of Protophyta, lying at the base of the vegetable kingdom, a nucleus has not, heretofore, been conclusively demonstrated. It would seem, from their varied mode of life, that these organisms are probably not only the lowest in the scale of life, but that they were probably the first to appear upon the earth when it was ready to support living organisms. No other class of plants can exist under such varied surroundings, though each species is most delicate in its response to its own environment. In the hot springs of the Yellowstone National Park, where the water reaches 190° F., the boiling point at that altitude, these are the only organisms that can find the necessary conditions of existence. They range from such conditions through all temperatures to the frozen seas of the North, and though the waters be strongly impregnated by alkaline salts or acids, the organisms are not yet excluded. It is just such environments that one would expect to find in the beginning of the earth's phytological history, and it seems reasonable to consider these organisms as the surviving basis of a phytological tree. Their power of elaborating food in the dark as claimed for the Cyanophyceæ by Hansgirg, and their ability to withstand long periods of drying which will shrink their cells to one-third of their natural size while still retaining their vitality, would also be in favor of their primitive origin.

There are many cytological problems locked up within the cells of the Cyanophyceæ, but in the past investigators have mostly confined themselves to the questions of the presence of nuclei and chromatophores. These still constitute the fundamental questions which must be confronted. Palla (60) thus sums up the situation in this respect: "We have three possibilities before us, either (*a*) the Cyanophycean protoplasts have true cell nuclei, whose significance we have not known up to the present time, or (*b*) the nuclei of the progenitors of the Cyanophyceæ have been reduced until they have entirely disappeared, or (*c*) the Cyanophyceæ are without nuclei." Though the present investigation does not confine itself to the nuclear side of the problems, nevertheless this phase of the subject must necessarily receive much attention. It was therefore with much interest that the subject in hand was entered upon at the suggestion of Professor Macfarlane, who has watched the different steps of the investigation, has verified the results and has made helpful suggestions from time to time.

The following sections make up the remaining part of this paper:

(1) Review of Literature, p. 240.
(2) General Morphology of Types Studied, p. 273.
  (*a*) Material used, p. 274.
  (*b*) Methods employed, p. 275.
  (*c*) The cell wall and sheath, p. 279.
  (*d*) The central body, p. 282.
  (*e*) The chromatophore, p. 289.
  (*f*) The granules, p. 292.
  (*g*) Vacuoles, p. 294.
  (*h*) Other cell-constituents, p. 294.
(3) Morphology of the Dividing Cell, p. 295.
(4) Protoplasmic Continuity, p. 299.
(5) The Heterocyst, p. 301.
(6) Spore Formation, p. 302.
(7) Motility, p. 307.
(8) Main conclusions, p. 323.
(9) General summary of results, p. 325.
(10) Bibliography, p. 327.
(11) Explanation of Plates XXIII, XXIV, XXV, p. 332.

(1) REVIEW OF LITERATURE.

The history of the work already done on the cytology of the Cyanophyceæ is one of conflicting opinion. The existence of a nucleus, for example, has been affirmed in the strongest terms by careful investigators, backed by seemingly conclusive experiments, only to be retracted later. Such views have been attacked by other equally capable workers, who as strongly denied the existence of a nucleus, and fortified their conclusions by apparently good evidence, sometimes even with the identical experiments used by the exponents of the nuclear theory.

Such conflicting evidence is the result, it would seem, of two factors: first, the want of any uniform meaning for the word "nucleus" among the different observers; and second, the failure to recognize that the nucleus and other cell contents may give different reactions with the same reagent at different times. This latter fact has been abundantly shown by the nuclei of higher organisms during division and other life phases, where varying concentrations and modifications of the nucleic acid (52) may occur. Why the same variability should, among the nuclei of the lower forms, be taken as indicating a different morphological structure is hard to conceive. The fact that protoplasm itself is a mixture of various complex bodies, which through metabolism and growth are continually changing, would leave no valid reason for considering that variations in the cell contents, even as profound as those of the "central body" of the Cyanophycean cell should not exist.

The earlier writings on the cytology of the Cyanophyceæ were by Schmitz in 1879. He did his work upon *Gloeocapsa polydermatica, Oscillaria princeps* and *Anabaena flos-aquae.* In his earlier work (67), after many attempts, he discovered a homogeneous central body which he considered to be a cell nucleus, though it did not seem to appear in every cell of all preparations. Hæmatoxylin showed this nucleus of *Oscillaria princeps* to be spherical and excentrically placed. He also found the cells to be more or less completely filled with large round granules of unknown composition, which he called "schleimkugeln." Though he used hæmatoxylin to stain his preparations, he was able to make out the structure equally as well without its use. He therefore discarded the stain and drew his conclusions from the fresh, unaltered material.

The following year Schmitz (68) again published on the cytology of the Cyanophyceæ. Here he retracted his views of the former year, declaring that these plants were non-nucleolated and non-nucleated. The central body, which he formerly considered to represent the nucleus, he now interpreted as very large microsomes which reacted like the nuclei of the higher plants. The peripheral protoplasm he found crowded with larger or smaller granules which stained deeply with hæmatoxylin like chromatin granules in other plants. This peripheral layer is what Schmitz busied himself with mostly in his second and third (69) papers, in the last of which he concluded that he was unable to find any specially formed nucleus or chromatophore in either the Cyanophyceæ or the Bacteria.

It is interesting to note that in his earlier work, Schmitz saw a homogeneous central portion which he called a nucleus. There was also a granular periphery to the cell which resembled a chromatophore. In his later works he still found a central body, but because it broke up into "microsomes," he no longer interpreted it as a nucleus, but as granules which were composed of nuclear constituents. His later works were given over to the study of the chromatophores more particularly. These he also failed to find, but attributed the function usually performed by such organs in the higher plant cells to the general protoplasm of the Cyanophyceæ.

Wille (85) worked principally upon *Tolypothrix lanata,* which he stained with hæmatoxylin, preferably concentrated. He could make out a dark blue nucleolus, which in nature is relatively quite large and colorless, and a pale blue nucleus in an unstained cytoplasm. The nucleus could also be seen in the granular cells when treated with dilute acetic acid. He also succeeded in demonstrating an intercellular continuity of the protoplasm in *Stigonema compactum, Ag.* At the time of cell division, which he was able to observe clearly, he found that each cell nucleus divided into two nuclei, each having one or two nucleoli. Thereafter the dividing cell wall was laid down. He did not claim a nucleus for all of the Cyanophyceæ, but regarded it as always present in the higher forms.

A criticism on the work of both Schmitz and Wille is that they used only one stain, hæmatoxylin, and in part of his experiments, Schmitz used only fresh material. The use of fresh, unchanged material is of course preferable if supported by other observations, but confirmatory tests are quite essential. Observational results without such safeguards are never conclusive though Wille's descriptions seem very accurate. His conclusions would have carried much greater weight if he had left figures illustrative of what he saw.

Tangl (78) was unable to find a nucleus, but this was probably due to poor methods. He described an Oscillarian organism, *Plaxonema oscillaris,* which had a rod-shaped chromatophore. However, as Molisch (55) showed phycocyanin to be a crystalline substance, Gomont (32) considered this chromatophore to be merely a crystallization of that pigment. Tangl described zoogloeæ which appeared on culturing *Plaxonema* as follows: "The filaments first lose their power of motion, coming to a standstill after the following changes, which are of two kinds (*a*) the filaments either separate into single cells, or (*b*) in certain places there arise small zoogloeæ which enclose a changing number of shorter or longer parts of filaments. . . . The plasma of the filaments assumes a finely granular appearance, the glittering granules on the partition walls alone remaining of the earlier contained bodies, the chromatophore becomes loosened and withdraws from the walls." From this description it seems hardly probable that the organisms were in a good state of preservation, and his conclusions for that reason scarcely bear upon the morphology of the Cyanophyceæ.

Borzi (6) worked on *Nostoc, Anabaena, Oscillaria, Spermosira, Cylindrospermum* and *Sphaerozyga,* and endeavored to determine intercellular protoplasmic continuity primarily, but his conclusions in other lines are as interesting, or more so, than in the line that he started to investigate.

He established the fact of such continuity, but at times the connecting substance might be composed of cyanophycin rather than protoplasm, as was the case with heterocysts, where it formed a plug to close the pore. He considered the connecting thread to be single and to pass through a pore in the middle of the partition wall. In *Oscillaria* this continuity assisted it to act as a unit in its movements. He was unable to demonstrate a nucleus or chromatophore. By means of micro-chemical methods he was able to demonstrate granules, partly imbedded in the protoplasm and partly applied to the wall (the "schleimkugeln" of Schmitz) formed of a gelatinous substance which, he believed, replaced starch in these forms, and which he termed cyanophycin. These granules were secreted by the transverse walls in dividing cells and had the following properties; the substance was homogeneous, they became swollen and disappeared when placed in sulphuric or hydrochloric acid, Millon's reagent gave no color to them though they were turned slightly blue by iodine (5) or chlor-zinc iodide. Isolated cells of *Oscillaria* viewed from the "end" showed that the granules were arranged in a circle around the central opening through which passed the connecting thread of protoplasm or cyanophycin. The cell wall and sheath were composed of a substance akin to cuticle. This sheath was very delicate in *Oscillaria,* and was often overlooked. It became thick over the rejuvenated ends of the filaments, forming thus a kind of protective cap. In spore formation, cyanophycin in the cell became the chief part of the spore and the cell seemed to rejuvenate. An encysting wall was then formed around the spore and the mother wall disintegrated.

After investigating numerous forms of *Tolypothrix, Oscillaria, Nostoc, Spirulina,* etc., Zopf (95) concluded that the filamentous forms were but one stage in the life-history of the "Spaltalgen." By growing filamentous forms on tiles imbedded in moist sand in such a way that very little nourishment could be obtained by them, he was able to cause the filamentous plants to assume the forms of short rods and coccus-shaped cells, thus proving that a much closer connection existed between the Cyanophyceæ and the Bacteria than had been hitherto supposed, even by Cohn or Sachs (66) who were the first to place the two groups together in the Schizophyta. Several forms as here described are doubtless produced when a filamentous alga breaks down in death, but it would, however, scarcely seem proper to consider as a part of the life-history of a plant, a phenomenon caused by an evident pathological condition. Methods quite similar to those used by Zopf were employed in the present investigation for the purpose of causing spore formation, with the result that the filaments would break down after forming spores. These may be the unicellular forms spoken of by Zopf. Concerning the structure of the cell, he says little, except to assert that a nucleus was present (96) in *Phragmonema,* but he was somewhat uncertain as to whether it should not rather be termed a vacuole.

According to Gomont (33) the cells and hormogonia of the Homocysteæ always possessed a delicate laminated membrane, which in the later formed and younger cells consisted of a substance closely allied to cellulose, but in the older cells it resisted all such reagents and he considered it to be a substance between the cuticle of the higher plants and fungus cellulose. The sheath of the Homocysteæ (31) ranged between hyaline and gelatinous, becoming hyaline for protection when exposed to light and air. It was distinctly laminated. The protoplasm was colored uniformly a blue-green, but had small granules of two kinds, one small, irregular in outline, refringent and collected usually at the ends of the cells, the other about 1 μ to 2 μ in diameter and supposed by Zacharias (89) to be composed of a hydrocarbon. The former were most abundant in the older cells, being scarcely shown in the growing tip where division is most abundant, and were entirely lacking in newly formed cells. Both kinds of granules could exist in the same cell, though either might be absent, and in portions of the trichomes where the cells were actively dividing, he observed at times a regular change from cells having granules to others destitute of them, and again back to the former condition. No chromatophore was found, that which had been described as such by other authors being a crystalloid. Nuclei could not be demonstrated, hence he denied the existence of indirect division. A central body, staining with hæmatoxylin, was found. Vacuoles were not present in the normal plants, and their contents when found in pathological material, were not known to him. Division of the cell was direct and proceeded very rapidly, often the "collar-like" ingrowth from the cell wall which caused division by strangulation formed for the granddaughter cells before the daughter cells were fully separated. He found the end cells protected by a calyptra, formed by the last cell ceasing to divide and forming around itself a hard coat. In the mature state, this calyptra was quite diagnostic for the species. Another striking feature of the end cells of certain Oscillarieæ was the presence of what appeared to be long sluggish flagella. These, however, he dismissed as not characteristic, and as being really parasitic growths, like bacteria, having no motion of their own. They did show a certain movement, he admitted, but he referred it to the oscillation of the trichome.

In 1888, Scott (71) exhibited some slides and reported the work of Miss H. V. Klaassen before the Linnean Society. The work was performed upon *Tolypothrix cooctitis* and three species of *Oscillaria*. He used two methods: (*a*) by treatment for five minutes in methylated ether, and then staining for four minutes in Kleinenberg's hæmatoxylin and mounting in balsam, or (*b*) by staining for two hours in picro-nigrosin and clearing for two minutes in chloral hydrate and mounting in pure glycerin. By either of these methods he recorded a rounded central body composed of fibrils, very similar to the skein stage of the karyokinetically dividing nucleus. This was considered to be a nucleus because it broke up into a number of pieces and the cell then divided, the ingrowing division wall being plainly seen. Between the daughter nuclei he could further distinguish, in some cases quite distinctly, fine colorless striæ which suggested the achromatic fibers of karyokinesis.

Probably the most extensive worker on the Cyanophyceæ was E. Zacharias, to whom we owe much in the solution of the cytological problems, not only of these but of all organisms. His researches covered not merely the staining relations of the different parts of the cell, but included an exhaustive micro-chemical study, and it is for this portion of his work that we owe him greatest thanks. His first investigation (88) published in 1887, was on *Tolypothrix* and *Oscillaria* and his methods and conclusions might be summed up as follows: *First.* Fresh material treated with gastric juice, was extracted with ether and alcohol, followed by 0.3 per cent. hydrochloric acid. The granules gave the characteristic "nuclein luster." *Second.* In material treated as above, followed by 10 per cent. sodium chloride or with 0.05 per cent. potassium hydrate the "central substance" disappeared. *Third.* Fresh threads treated with 55 per cent. hydrochloric acid caused the "central substance" to swell. These considerations caused him to conclude that the "central substance" was a nucleus.

In a later work, Zacharias (89) extended his studies to include, besides the former organisms, *Nostoc, Tolypothrix, Cylindrospermum* and *Scytonema.* It was in this work that he took up the micro-chemistry most fully. He found a colored peripheral layer, often thickest on the partition walls, which he thought was surrounded by a thin uncolored plasma layer, and a central part which always remained clear. He could not determine whether the colored portion was to be regarded as a chromatophore or not. In the central part he saw many granular frame-like formations and one or two nucleolar-like bodies, though they were not present in all cells. A portion of the central body he found to be soluble in artificial gastric juice, the insoluble portion being composed of two substances, one related to plastin, which was always present, and the other, which he called "central substance," was allied to nuclein of higher plants. This might be absent, especially in dividing cells where it seemed to disappear. In discussing the relationships of the "central substance" to the nuclei of higher plants, he concluded that it differed in all respects from a true nucleus, basing his inferences, among other reasons, principally on the following two foundations: (*a*) Nuclein might or might not be present in all of the cells of the Cyanophyceæ, or it might be present in some and absent from other cells of the same trichome; (*b*) Nuclein disappeared entirely from the dividing cells of the Cyanophyceæ, while division in the higher plants was always preceded by an increase in the amount of chromatin. He was of the opinion that the absence of sexuality in these organisms was related in some way to the absence of a nucleus. Zacharias called the slime balls of Schmitz "grains," and could not identify them as paramylum or as a gelatinous substance, but after exhaustive micro-chemical studies he concluded that they were of a carbohydrate nature. In division he described a collar-like ingrowth which constricted the cell into halves, but he could find no evidence of karyokinetic figures. After continued cultivation of the Cyanophyceæ in his laboratory, he observed that the "central substance" or so-called "nucleus" became smaller and finally disappeared, and would again as mysteriously reappear. He could not account for this behavior.

In another paper (91) called forth by the works of Hieronymus and Zukal, Zacharias reaffirmed his results as outlined in his work of 1890. In regard to a chromatophore, he found, in properly prepared living *Scytonema,* a peripheral plasma pierced by minute pores. Small colored bodies seemed to be imbedded in the cytoplasm which surrounded a colorless foundation-mass. In 1900 Zacharias again published the results of an investigation of much moment. In it he discussed at some length the staining theories of Fischer in a manner that brought out many interesting points. In fact Zacharias has published several (92, 93) very able articles on methods of cytological investigation which have been of great value in the present inquiry. His conclusions were that while there were restrictions to be held in mind, still it was quite admissible to use staining reactions in determining the cell and nuclear constituents. In this work, Zacharias, in discussing at great length the cell contents of the Cyanophyceæ, concluded that "from the present state of our knowledge we can say in regard to the arrangement of the colored and colorless contents of the cell, that a colorless central body is surrounded by a colored plasma. The latter may be lacking under certain conditions," but as there was no bounding membrane, he would not call the colored portion a chromatophore. The central body was a very irregular, structureless and compact mass, enclosing larger or smaller colorless spaces. Its contents showed great varieties of condition, even in the same species. He said, "As many different species of *Lyngbya* as are in the palm house at Hamburg, each one showed a different arrangement in the cell rows." The same species also differed at different times of the year. The granular constituents he considered to be of two kinds, the cyanophycin granules in the peripheral plasma, and the colorless central granules embedded in the periphery of the central body, which corresponded to Palla's slime balls, though he did not use that term on account of the different meanings given to it by different authors. He considered it quite possible that his "central granules" were composed of chromatin though he was unable to demonstrate it to his satisfaction. When treated with 28 per cent. hydrochloric acid they had the appearance of hollow balls. They occurred most abundantly in the spores and not at all in the heterocysts. It was quite possible, he thought, that a delicate connection existed between them and the central body, "but," he continued, "we cannot decide with certainty whether the central substance of the Cyanophyceæ corresponds or not to the cell nucleus of other organisms."

Bütschli's investigations (7) extended over the Bacteria and Cyanophyceæ, especially such forms as *Ophidomonas Jenensis, Chromatium Okenii,* and other forms of bacteria and spirilla from marsh water, and several forms of *Beggiatoa, Cladothrix, Oscillaria* and *Nostoc,* all of which he repeatedly investigated, and always found the cells of all forms corresponding to the same general type. This type consisted of a bounding membrane or cell wall, which he regarded as a plasma structure. Within this was the chromatophore, a thin colored rind-layer of plasma having a netted or honeycombed structure and surrounding the colorless "central body." In the bacteria the central body was not demonstrable in all forms, but when it was present it was colorless and drawn out, and the surrounding zone was accumulated at its poles. On staining with hæmatoxylin he found the "central body" to be netted, or with a coarser honeycombed appearance (Wabenstruktur). At the nodal points of the web-structure, in material killed in alcohol or dried, he found granules which stained a red-violet with weak hæmatoxylin. They were very abundant and even jutted out into the surrounding chromatophore and sometimes were found isolated in the surrounding crust layer, but when so found, were always small. These he termed "red granules" on account of their staining properties with hæmatoxylin. He could not stain them thus in material that had been killed in corrosive sublimate, osmic acid mixtures, or picro-sulphuric acid. They stained intensely green with acetic acid methyl green. After treatment with artificial gastric juice, they no longer stained characteristically with the hæmatoxylin, but he did not think that they were digested out, but had merely lost their staining properties. He denied their identity with the cyanophycin granules or "schleimkugeln" of other authors, and with Nadson considered them to consist of chromatin. This view was further strengthened when later (9) he found these granules in the cytoplasm of other organisms as Diatoms, Flagellates, etc., where they have an undoubted chromatin nature. He also demonstrated the "schleimkugeln" of Schmitz in great abundance along the partition walls of *Oscillaria.* They were stained by eosin, but were not affected by hæmatoxylin. In this respect he contradicted Zacharias (91) who was able to demonstrate them only by the use of hæmatoxylin. If *Oscillaria* was subjected to artificial gastric digestion, the peripheral layer was dissolved out completely or nearly so, but the "central body" remained and appeared more like a nucleus than ever, retaining its power of staining with hæmatoxylin. Micro-chemically, he found the "schleimkugeln" to contain much iron, and glycogen seemed to be the form of the reserve food. From these observations, Bütschli based a strong argument for the nuclear character of the "central body," which he held to divide by direct division.

In the following year Fischer (26) attacked this view, claiming that Bütschli's methods of fixation were faulty and caused plasmolysis, thus forming an artifact in the cell which was mistaken for a definite peripheral layer or chromatophore. Fischer's observations were based upon the study of an unspecified species of *Oscillaria* and of Bacteria. In these, by methods similar to those used by Bütschli, he got a plasmolysis which left strands of protoplasm attached to the cell wall and such, he thought, Bütschli, had mistaken for a web structure in the so-called chromatophore. He did not consider the "central body" as described by Bütschli to be nuclear in its affinities, but rather that it was the main mass of the shrunken protoplast. He affirmed that a nucleus was present, but that it was to be looked for within the central mass of the protoplasm which Bütschli termed the "central body" or nucleus. Fischer acknowledged that the "red granules" described by Bütschli were present, but that they had been known for a long time and could be seen in the living cells, though he did not consider them to be of any morphological value. Fischer, however, failed to tell where they were recorded prior to Bütschli's description, and in as extended a search as could be made in the present investigation, it has been impossible to locate any reference to them of an earlier date.

To these objections of Fischer, Bütschli (8) observed that some reagents did produce plasmolysis, but this was not the usual case, and when properly handled, it did not occur at all with the reagents used by him, which were Flemming's chrom-osmic acid mixture, picro-sulphuric acid, with and without having had osmic acid added to it, and osmic acid alone. He fortified himself behind the fact that most of the recent investigators had observed the colorless "central body" in the Cyanophyceæ, and concluded that Fischer's criticism was based upon unfinished and hasty observations. In another work (27) based upon much more extended studies of the structure of Bacteria, Fischer reaffirmed his former position, and objected still more strongly to the methods and conclusions of Bütschli, who thereupon published the results of two very carefully performed investigations (9, 10) in which he reaffirmed his former views, enlarged and fortified his position, and was able to demonstrate the comb-like structure in the "central body" of the living cell, though he pointed out differences in one and the same species. Beyond this he added very little to his former papers except to refute the conception of the "red granules" as held by Kunster (44) and Busquet.

Hieronymus (41) studied the Phycochromaceæ and concluded that a nucleus was present. It was composed of granules upon a thread very similar to the spirem stage of division in higher plants. These nuclear granules contained a substance which represented, and performed all of the functions of the chromatin, but was not that substance. No nuclear wall was present, hence he suggested that the nucleus be called an "open nucleus" in distinction to the "closed nucleus" of the higher plants. In cell division the cell wall grew inward, gradually constricting the spirem-like nucleus until it was finally separated into two parts. A chromatophore was also described, consisting of small granules of chlorophyll joined together like a string of beads, and ramifying through the outer cytoplasm in which the blue coloring matter, phycocyanin, was dissolved. These chlorophyll grains were connected with the skein-like nucleus by similar threads. This observation is very interesting in the light of the recent work of Watson (81) performed in this laboratory. Watson has shown that the plastids of higher plants are probably derived from the nucleus and are connected with it by protoplasmic threads, but whether the observations of Hieronymus can be looked upon as an earlier type of what Watson has found in the higher plants remains for further investigation. Hieronymus considered the granules of the cytoplasm to be the "cyanophycin granules" of Borzi, or what were denominated "granules" by Zacharias and "red granules" by Bütschli. These granules he considered to be neither reserve food nor the products of assimilation, but that they were unwound from the "central body." They were crystalloid and angular, crystallizing after one of the regular systems, according to their chemical composition. Immediately within the cell wall was a thin hyaline layer enveloping the protoplasm. In answer to a criticism by Zacharias (91) Hieronymus (40) still maintained that there was but one kind of granule in the Cyanophyceæ and attributed any others to faulty manipulation. He was able to stain all granules alike with hæmatoxylin, if he used ammonia afterwards. This treatment, however, would seem scarcely permissible for the reason that the red staining reaction of granules by means of hæmatoxylin might be due to the presence of an acid, which fact in itself would show a difference between granules. This acid would, of course, be neutralized by the ammonia, and the granules would therefore stain similarly to others which did not need this neutralization, and for that reason were different granules. Hieronymus was able to demonstrate that vacuoles were normally present in the central bodies as well as the peripheral cytoplasm, and were even found in the end cells of young growing filaments. Though others had found vacuoles in the cytoplasm, Hieronymus was the first to claim them for the nucleus. These vacuoles varied in size from very minute points to more than one-half of the size of the cell, as in *Scytonema circinatum* and *Stigonema ocellatum* of Thuret.

Palla (60) worked upon a variety of organisms, making very exhaustive experiments. His results may best be given by a translation of his own concluding paragraphs. He says: "These are the results of my investigations thus far: (1) The protoplast of the Cyanophycean forms investigated always showed a differentiation into a colorless part, the 'central body,' and a colored crust layer, the 'chromatophore,' outside of which was a colorless ectoplasm, and inside of which, between it and the 'central body,' was a colorless plasmatic layer, the 'plasmaschicht.' (2) In the case of *Gloeotrichia pisum,* and probably of other Rivulariaceæ, there are several 'central bodies' in most cells, and they appear in the most dissimilar formation in the same cell, while the other Cyanophyceæ have generally only one 'central body' in the cells. The central body is homogeneous and has a structureless membrane surrounding it. It divides by constriction into halves. It is characteristically stained by intra-vitam staining with methyl blue. (3) The chromatophore has a vesiculated structure (webenbau of Bütschli), the coloring matter never appearing uniform, but bound up in numerous small carriers, which are not pure chlorophyll green, but possess the color in which the chromatophore appears as a whole. (4) Large vacuoles are a normal structural appearance in the Cyanophycean cell. They are generally to be seen in all Cyanophyceæ, and in *Gloeotrichia* and many other Rivulariaceæ they are a constant feature. (5) The granular bodies which appear in the Cyanophycean protoplast, are always observed outside the 'central body.' According to their reactions, they are divided into two widely different groups, the 'cyanophycin granules' and the 'slime balls.' The cyanophycin granules, which under ordinary conditions apparently consist of a firm substance, are easily soluble in dilute hydrochloric acid, are colored blue by hæmatoxylin, and when stained intra-vitam, retain no methyl blue. They are generally found in the outermost part of the periphery of the chromatophore, and are doubtless to be regarded as the first visible assimilation product of the activity of the chromatophore. In the spores are placed the necessary reserve materials for their germination. The slime balls, which are composed of a tenacious, flowing substance, are insoluble in dilute hydrochloric acid, are colored red-violet with hæmatoxylin, and store up methyl blue during intra-vitam staining. They are closely placed around the central body, and only occasionally appear further out in the chromatophore. Their significance in the cell is not clear. The 'nucleolus,' the 'central substance' and the 'red granules' of other authors are bodies identical with the slime balls. (6) In the germination of *Gloeotrichia* spores, oil appears in the chromatophores of the cells. Zukal had also noticed this in other Cyanophyceæ. It can be established conclusively that the Cyanophycean protoplast is made up of at least two different parts, sharply distinguished from one another, and it is questionable whether we should call the central body a cell nucleus." Following this he gave a somewhat lengthy discussion of the phylogenetic relationship of the central body to the cell nucleus, and he concluded that on account of the "lack of a chromatin framework, the absence of nuclei, the direct division which may be more complicated than it appears to be, the central body was widely different from the cell nucleus, though the two might probably have originated from a common organ."

Marx (53) confined his attention mostly to micro-chemistry, and from this standpoint his paper is of considerable interest. Morphologically he concluded that the cells seldom showed a central body. He could stain the granules of the cells black with osmic acid and concluded that "in spite of the many repeated attempts, it is not possible to determine the existence of a cell nucleus."

Deinega's observations (21) were carried out on *Oscillaria princeps, O. Froehlichi, Aphanizomenon flos aquae, Nostoc* (sp.) and *Scytonema* (sp.), and were directed toward solution of the problems presented by the granules, the nucleus and the chromatophore. He found a chromatophore in the form of a plate lying next to the inner wall of the cell and running parallel to the longitudinal axis of the trichome, the coloring matter being in trabeculæ. He found granules of only one form which were grouped along the cell wall in *Oscillaria,* and which, from staining reactions and micro-chemical tests, he considered to be an isomer of starch, but not paramylum as held by Cohn (16) and Hansgirg (34). He was able to stain the central body more deeply than the surrounding cytoplasm and considered it to be composed of a conglomeration of granules, but he did not assign to it the function of a nucleus, leaving this question open because he could not demonstrate the central body in all cells by the use of hæmatoxylin and other reagents. He attributed the "nuclein luster" caused by digestion in artificial gastric juice to the remains of the chromatophore, and supported his work by check experiments on *Spirogyra.* The appearance which Scott interpreted as cell division, he considered to be an artifact caused by the swelling of the cyanophycin grains along the cell walls which thereby gave the appearance interpreted as division. Zacharias (90) in an objection to these views, showed definitely that the conclusions concerning *Spirogyra* were based upon very faulty and insufficient observations of that organism.

Zukal (100), working on *Tolypothrix lanata*, concluded that the structures which Wille (85) termed "nucleoli" were in reality a number of small cell nuclei, each surrounded by a portion of cytoplasm, having arisen endogenously by division of the cell nucleus, but having no wall. There might be as many as sixty-four of these present, all connected by a network with the central body. These he termed the nuclei, founding his conclusions on their power to divide. He cited, however, his difficulty in getting constant results, but this, as has been pointed out above, should scarcely be considered seriously, except that care must be taken, by the use of several reagents, as there suggested, to avoid the formation of artifacts. In a later work (99) Zukal again called attention to these granules, which he now identified with the "red granules" of Bütschli. They stained very lightly with safranin, eosin or hæmatoxylin, but hot methyl blue brought them out very strongly. They did not really disappear when treated with 5 per cent. potassium hydrate, chloral hydrate or 1 per cent. hydrochloric acid, but swelled more or less and seemed to disappear. He concluded that the granules were coagulated albumen, which formed the cell nuclei and surrounded the central clear mass of cytoplasm. He did not enter minutely into the question of cell division or of the nucleus, but his conclusions on this point were as follows: "In each cell nucleus two nucleoli are formed after each simple (amitotic) division. This occurs mostly at night. The division of the nuclei ('red granules' of Bütschli) may proceed without the division of the cell." In regard to the chromatophore, his definition was, "a definite, demarcated part of the protoplasm, saturated with the characteristic coloring matter of the plant." He considered such a chromatophore, formed of an exceedingly delicate reticulum of fine granules, to be present surrounding the colorless cytoplasm of the Cyanophyceæ. Still later Zukal

(102) reaffirmed a part of the above observations, but modified his view concerning the distinction between the central cytoplasm and the chromatophore. He now considered that there were two forms of granules, the cyanophycin granules of Hieronymus, and the slime balls of Palla, the former being connected with the central body by means of threads in their earlier stages. At certain times, quite simultaneously, the cyanophycin granules would lose their cyanophycin, and become slime balls and *vice versa*. When the cyanophycin was extruded into the cell, it might crystallize in the cytoplasm or it might disappear. These granules had a particular protoplasmic framework and were organs of the cell. The slime balls could flow together to form a central mass, which became the "central substance" of other authors (Hieronymus and Zacharias). This central substance might become rounded off, with a drop of cyanophycin in its centre, when it resembled a cell nucleus. The central substance might break up in two ways: either by formation of a large number of fine granules suspended in chains which finally separated and became slime balls by growth, or the central body might divide into 2, 4, 8, etc., bodies which became the slime balls. These, finally, might take up cyanophycin and become cyanophycin granules. This explained why slime balls and central substance, or slime balls and cyanophycin granules were seldom seen together in the same cell. In the same year (1894) Zukal published the results of another investigation (101) in which he held that the chromatophore had a webbed or fibrillar structure. He reaffirmed the general points of his former observations, but augmented them. He now found that the youngest cells were colorless and had no webbed chromatophore, which was a later development. It segmented out of the cytoplasm, dividing the cell into smaller portions or units. The granules were formed by certain points in the fibrils which gradually enlarged until they became granules similar to the "red granules" of Bütschli. These granules represented

the nucleus and might change from one form to the other. By far the most interesting portion of Zukal's work, however, consisted in the finding of very small motile zoospores of two sizes, which he called "gametes." These underwent a process of conjugation, a large one uniting with a small one, the two surrounding themselves with a common gelatinous envelope, though he was not sure that they fully fused. They finally began to divide by cross partitions and then grew out into new plants. Both sizes of gamete were formed within the protoplasm of the cell, and upon the rupture of the cell wall they floated out into the water where conjugation occurred (Fig. 65). In 1899 Zukal (103) carried out additional work on the Cyanophyceæ, from which he concluded that the cyanophycin granules changed to slime balls, which in turn passed to the centre and fused into a spider-like body which lay along the partition wall between two cells. The substance of the spider-like mass was a soluble modification of the granules, and bore about the relation to them that grape sugar does to starch. It travelled from cell to cell through pores in the partition walls in order that it might increase at certain points, as in the akinetes. In 1894, in his work (101) on *Oscillaria,* he considered that the colored crust layer was a chromatophore. The vacuoles, especially in the hair-like ends of the Rivulariaceæ where they appeared regularly, he thought to be signs of degeneration. He also thought the central substance to be identical with the slime balls of Palla.

If Zukal's contentions are correct, it would seem that the three negative characteristics of the Cyanophyceæ, *i. e.,* lack of nucleus, lack of chromatophore and lack of sexuality, have all been proven to be erroneous. However the possibilities of making such observations seem quite improbable, especially in living material arranged in "hanging drop" cultures as he used. It is quite impossible to use the exceedingly high powers of the microscope that are required to show the structure and conjugating gametes on account of the depth of the drop, which would carry the plant beyond the focal distance of the objective. At least this has been the difficulty experienced in trying to follow Zukal's observations on living material in the present investigation.

Nadson's work (57) was principally performed upon *Merismopedia elegans, Gloeocapsa polydermatica, Lyngbya curvata, Oscillaria* (several undetermined species), *Aphanocapsa Grevillei, Chroococcus turgidus, Tolypothrix ægagrophila, Aphanizomenon flos aquae,* and for comparison, the bacteria *Clostridium butyricum* and *Cladothrix dichotoma.* For the purpose of fixation he found iodine-alcohol the most useful, also a saturated aqueous solution of corrosive sublimate. Hæmatoxylin was his chief stain, though vesuvin, iodine green, a mixture of methyl blue and fuchsin, and acid carmine were also used. Intra-vitam staining with methyl blue was also productive of good results. Most of his conclusions on fixed material he was able to verify on living specimens, thus obviating the possibility of mistaking artifacts for normal structures. Micro-chemical reactions did not give him any definite results and were therefore discarded. A colorless, amœboid central body, occupying most of the cell, was found, distinguished peripherally by phycochrome from a colorless outer layer, though this latter was not to be regarded as a chromatophore, but rather as protoplasm. In one form (*Aphanizomenon*) vacuoles were found between the central body and the protoplasm. The cytoplasm had a vesiculated structure like the "wabenbau" of Bütschli, and the chlorophyll and phycocyan were contained in the walls of these vesicles. The central bodies were not always surrounded by the protoplasm, but in some filamentous forms (*Tolypothrix, Aphanizomenon*) they reached from end wall to end wall, thus forming a longitudinal band along a row of cells. The central body had the same vesiculated structure as the protoplasm, but was not so well defined, the vesicles being filled with a peculiar, deeply staining substance which the author called "filling substance" (füllsubstanz). Three kinds of granules appeared in all cells. One form, found mostly, though not entirely, in the central body and corresponding to the "red granules" of Bütschli or slime balls of Palla, he called "chromatin granules" because in their reactions they showed the same characteristics as the chromatin of the higher plants. In size and number they varied greatly, but this variability did not affect their power of division, which took place through their arranging themselves in form of the figure "8." Each constricted later into two nuclei, caused by the ingrowth of a collar-like band from the lateral walls of the cell. To the second class of granules, corresponding to the cyanophycin granules of Borzi and Palla, he gave the name of "reserve granules," considering them to be composed of reserve food akin to the starch of the higher plants. They were only found in the protoplasm, usually along the partition walls, and their number was very variable according to the condition of nourishment of the plant. They were most numerous in the spores. The third form of granules of a plasmatic structure, were denominated "microsomes." They occurred at the nodal points of the vesicles of the protoplasm. No reason was assigned for believing them to be of a different structure from the protoplasm, other than that they had a slight tinge of color. The large bacteria he considered to have a structure similar to that of the Cyanophyceæ, but the smaller forms, which Bütschli and others considered as having only the central body without protoplasm, he thought to have an undifferentiated protoplast which corresponded to the central body and the protoplasm combined. To this form of cell content he gave the name "archiplast," retaining the name "protoplast" for differentiated cell contents as formerly used. Chromatin granules were often present, but strewn through the whole archiplast. His conclusion was that the central body was very similar to the nucleus of the higher plants and doubtless represented it, for when the organism was doubly stained with methyl blue and fuchsin the protoplasm and the web structure of the central body were "erythrophil," while the "filling substance" of the central body, and the chromatin granules were "cyanophil," but on account of what he termed the "changeableness of its morphological characteristics" he doubted whether *all* cell nuclei of higher organisms were derived from the central body, though he regarded it as the philogenetic forerunner of the nucleus.

Zimmermann (94) gave a critical review of the opinions of the different authors concerning the Cyanophyceæ, and showed that there existed great confusion regarding the granular constituents of the cell. This he attributed to false identification of the granules. He identified the chromatin granules of Nadson with the red granules of Bütschli, and with the slime balls of Schmitz and Palla, while Nadson's reserve granules he considered to represent the cyanophycin granules of Palla. He retained the names "chromatin granules" and "reserve granules," and considered that the former represented a definite nucleus, which he found to be insoluble in dilute hydrochloric acid, to stain a red-violet with hæmatoxylin, and to take up methyl blue by intra-vitam staining, while the latter were soluble in dilute hydrochloric acid, stained blue in hæmatoxylin, and did not take up the color by intra-vitam staining. Division he considered to be direct by mere constriction of the cell contents, and thus showed no similarity to karyokinesis.

Macallum (49) in 1899 wrote a somewhat lengthy article on the cytology of the Cyanophyceæ, in which he recorded some very interesting experiments, especially micro-chemical, but his results as given below were mainly negative. In the living cells of the Cyanophyceæ he found two zones demarcated, but easily discernible; one central, denser and uncolored, and an outer peripheral one, containing the pigment which was dissolved in a fluid contained in vesicles. There was no evidence of a special chromatophore. The central body was finely vesicular and, except in its periphery, almost free from granules. A small quantity of a chromatin-like substance was found in it, which resisted digestion with artificial gastric juice. This material contained phosphorus and "masked" iron, and was uniformly diffused throughout the cytoplasm of the central body. The protoplasm of the peripheral layer was always more coarsely vesiculated than the central body. There were usually two types of granules present, one set which stained with hæmatoxylin, and contained "masked" iron and phosphorus and therefore resembled chromatin, but which became dissolved by artificial gastric digestion. These, which he called "granules of the first type," were hollow when they became large and divided at the time of cell division. They were usually found in the peripheral part of the central body, though they might extend to its central part or to the inner portion of the peripheral cytoplasm. The "granules of the second type" were found in the outer protoplasm and chiefly adjacent to the cell membrane. They rarely took the form of hollow spheres. They stained deeply with picro-carmine, had no organic phosphorus or "masked" iron, and dissolved very quickly in weak acids. They were probably of a proteid nature. The heterocyst was a degenerated cell in which the distinction between central and peripheral parts was lost. The chromatin-like substance of the central body diffused throughout the cytoplasm when the heterocyst was formed. When fully developed, the cytoplasm gave a feeble reaction for iron. A small mass at one or either pole of the cell gave a distinct reaction for "masked" iron and stained deeply with picro-carmine. As it did not dissolve in acids it was not related to the granules of the second type. He thought that the formation of the heterocyst next to the spore in *Cylindrospermum majus* and other forms, as well as its development beside the cells out of which arise the lateral branches in *Tolypothrix,* would appear to suggest that it might be the result of some rudimentary sexual process. He believed that there was no cell nucleus nor any structure resembling one in the Cyanophyceæ. Division was direct, the central body first showing the effects of the process. When a large spherule of chromatin-like substance was present, it might pass into one daughter cell or it might be mechanically divided between the two daughter cells. It seems that some, at least, of Macallum's conclusions are scarcely warranted by his experiments. He found that there was a chromatin-like substance present, still he denied the presence of anything resembling a nucleus. Were we to take the predominance of authority to determine a definition of nucleus, we must let nuclein or nucleus stand as fairly synonymous with chromatin. Where one is found, the other is indicated, at least indirectly. Macallum's error, like that of many others, seems to be in the mistaken idea that a nucleus must be a definitely bounded body.

Strasburger (76) claimed that there were special cells, no longer capable of division, in which several nuclei had been formed by fragmentation. The cell nucleus was surrounded by a colored peripheral layer or chromatophore in which cyanophycin grains were found. The function of these grains was unknown. Mucous globules were also disposed in the vicinity of the nucleus, and vacuoles were occasionally present. The cell wall was composed of cellulose, often distinctly stratified, and its outer layer might undergo mucilaginization. Multiplication was effected in a vegetative manner, *e. g.*, in the Oscillariaceæ, by the rounding off of two adjacent cells to form a germinal segment. The whole filament might break up into short hormogonia, each of which then grew out into a long filament. He accepted Hegler's conclusions of a mitotically dividing nucleus.

Bornet and Flahault (4) considered that the sheath of the Cyanophyceæ received the color which it often possessed through the action of light and air. Working upon *Tolypothrix lanata,* they were unable to discover anything akin to a nucleus. Bornet (3) investigated also the structure of the cells of Nostoc in lichens, and noted that their contents were much more fluid and homogeneous than those of the corresponding algæ not thus symbiotically related. Palla (60) also spoke of the absence of cyanophycin granules in the gonidia of lichens.

Warming (80) found a cell nucleus, and said that the coloring matter permeated the whole of the protoplasm except the nucleus, but in a few forms (*Glaucocystis, Phragmonema*) slightly developed chromatophores were present. Cilia were wanting, but the filaments were sometimes self-motile.

Chodat and Malinesco (14) distinguished only one kind of granule in *Cylindrospermum* and *Tolypothrix,* but such granules were more abundant in the younger cells. They said: "In the young cells they are differentiated in their protoplasm. In the adult cells the maximum development is reached. In the older filaments and those surrounded by a thick wall they diminish greatly in number." Chodat (11) in working on *Chroococcus turgidus,* concluded that the central body arose from the vacuole or emulsion-like appearance of the central part of the protoplasm, because the foundation substance of the central bodies usually stained like the peripheral protoplasm. It also had the same coloring matter and could not be easily distinguished from a chromatophore. He did not distinguish between slime balls, soluble starch, and cyanophycin, which he found distributed either uniformly or only in the central body. He also found glycogen. In division the separating wall was protoplasmic and stained the same as the other protoplasm. It could not be distinguished from that substance until later, when it became a true cell wall. He therefore questioned strongly the existence of a colorless central body and a colored crust layer. Later Chodat (12) was able to stain the cyanophycin granules and the central body a ruby color by means of methylene blue, while still later, in a note published jointly with Goldflus (13) they described "pseudo spores" in the Cyanophyceæ, which were "completely stuffed with cyanophycin."

Vines (79) claimed that the cells of the Cyanophyceæ were nucleated, but that the chlorophyll and phycocyanin were diffused throughout the cytoplasm and not aggregated in special plastids. Motion was found in some of the Cyanophyceæ, but its mechanism was not understood. Stockmayer (74), in an advance notice (the completed work of which apparently has not yet appeared, though several years have elapsed), took about the same ground as Palla concerning the cell contents, though he claimed no new points over other writers to support his thesis. The granules lay in the peripheral protoplasm and not in the central body, which he considered to be homogeneous, except for a web-like structure as claimed by Bütschli. According to Langerheim (45), who worked on *Glaucocystis nostochinearum,* no nucleus was present. In the younger cells, a chromatophore occurred in the form of a thread passing through the central part of the colorless portion of the cell, but in the older cells it changed its form to that of a granular membrane enclosing the colorless portion, and was some distance from the cell wall. Reinhardt (65) worked upon *Oscillaria major* (?), using picric acid as a fixing agent, and hæmatoxylin for staining. He saw very large granular nuclei, the granules of which he termed nucleoli, but he could not find the nucleus in all cells. The protoplasm had large and small granules, the former of which he termed chromatophores. Division was effected by a constriction of the protoplasm through the ingrowth of a ring-like collar which finally became the dividing wall. The work of Ernst (23) was carried out mostly on bacteria that were forming spores, *i. e.,* on starved cultures. He found that by staining with methylene blue and Bismarck brown he brought out certain small blue spore-like bodies, which appeared before the spores were formed and which he considered to be of the nature of nuclei, *i. e.,* composed of chromatin. The spores were formed by a direct metamorphosis of these bodies, on account of which he called them the "spore-producing bodies." By treating *Oscillaria* with the above stains, he found in each cell a collection of rounded black granules which reacted like the "nuclei of the bacteria." These granules surrounded one or more large drops in the middle of the cell in such a manner that the more delicate granules were at the periphery of the cell, thus giving the nucleus the appearance of a collection of round grains of chromatin which stained with hæmatoxylin similarly to his bacterial nuclei. Division was direct. Hansgirg (37) investigated the formation of glycogen in the plant cell, and found that this substance was normally present in *Oscillaria* (34). In working upon the question of the chromatophore and cell nucleus, he studied (36) *Chroodactylon Wolleanum, Gloeotheca* and other forms, and concluded that there was a sharply marked cell nucleus and chromatophore in each cell of the unicellular forms, but that in the thread forms, the general protoplasm discharged the function of both these organs. He saw the granules which Schmitz called "slime balls," and found them to be soluble in concentrated sulphuric acid and in a 10 per cent. solution of potassium hydrate. They were not stained by iodine or hæmatoxylin in the same manner as the surrounding protoplasm and he thought them to be paramylum granules. Hansgirg's figures are not clear, and leave a great deal to be guessed at by the reader, but the fact that the unicellular forms were found to have the nuclei and chromatophores while the filamentous forms did not, though treated in the same manner, would lend color to the contention of Bornet and Flahault (89) who did not consider *Chroodactylon Wolleanum* as belonging to the Cyanophyceæ. Goebel (30) stated that there was no nucleus in the Cyanophycean cell, but with Schmitz he considered that there were granules of nuclear matter scattered throughout the cytoplasm. The cell wall, when swollen, showed distinct stratification. It frequently deliquesced and became a thin jelly, in which the cells lay scattered.

Fischer (28) devoted considerable space to proving that no reliability could be placed in stains for the differentiation of cell constituents. He considered the staining reactions to be wholly physical, and due to the power of a substance to absorb the stain, though just what the physical properties were, as distinguished from chemical, which caused this absorption, he did not state. It would seem that, if two portions of a cell stained differently with the same stain, or if the same portion stained differently at different times, there certainly must be some difference, chemical or physical, which would cause the different reactions. If, then, there be such a difference in the stainability of the cell parts, it would scarcely seem proper to simply sweep all such reactions aside as worthless. It makes little difference whether the reactions be caused by chemical or physical processes, so long as a difference in cell structure is shown. After discarding all staining relations, Fischer proceeded to study the cell contents of the Cyanophyceæ and Bacteria. By digestion in hydrofluoric acid he found that all of the cell contents were dissolved except the chromatophores, which were usually hollow and barrel-shaped or cylindrical, according to the form of the cell. He could not make out any pellicle of protoplasm surrounding the chromatophore, but inferred that there was one from the fact that the granules accumulated on the partition walls. These granules he thought to have no significance as cell organs, rather they acted as assimilative and reserve products, the nature of which, whether albuminous or carbohydrate, it was impossible to determine. He maintained that "the ground mass of the central body is nothing more or less than a main part of the protoplast surrounded by the chromatophore in which the assimilation products are imbedded. The central body was in no way the prototype of a nucleus, and the cell had no organ in any way like a nucleus, either phylogenetically or otherwise. This central body might be surrounded by a very delicate membrane that ran from partition wall to partition wall. At the time of division, the granulations showed no characteristic groupings and that which was taken by some to be mitotic division was merely made up of aggregated crystalloids. Division was accomplished by an ingrowth of the wall, which cut the chromatophore and then the central body, but the central body itself did not, *pari passu,* begin to divide as in direct division of the higher plant nucleus. It was merely constricted by the ingrowing peripheral wall until a slight tag of protoplasm remained to connect the halves. This finally separated and division was complete. The central body was vacuolated and the pressure of these vacuoles caused the granules of the central body to become heaped up. Concerning the bacteria, Fischer considered the claim that they absorbed nuclear stains to be a myth. The central body described by Butschli as having clear ends he considered to be nothing more than the plasmolized protoplast. He found in these organisms, within the cell wall, first a protoplasmic wall-facing that surrounded a sap vacuole. A nucleus was not to be seen, the colored granules being reserve food material within the cell. The relation of the sulphur and other bacteria to the Cyanophyceæ was very loose and mostly morphological.

Probably the most exhaustive work that has yet appeared. and certainly the one which commanded greatest interest on account of the advanced ground taken was by Robert Hegler (38). The conclusions drawn in this article were so far-reaching, and in many respects so exhaustive, that it seems best to give his results at some length, substantially as summed up by himself in his concluding remarks, though adding some points to make them more clearly understood. (1) The cells of the Cyanophyceæ are in all cases surrounded by a cell membrane of peculiar material, distinct from the sheath, which latter is identical with the capsule of the encapsulated bacteria. Naked cells do not appear in these plants. The hormogonia do not possess a *demonstrable* membrane. (2) The gelatin coverings and the sheaths are the products of the membranous walls. The sheaths as well as cell membranes have a marked power of withstanding chemical reagents, and therefore resemble the cutin of the higher plants, but in chemical and optical characters have no relation whatever to it. (3) The walls of the heterocysts, on the other hand, consist wholly of cellulose. (4) A substance which has the nature of pectine (staining with ruthenium red) is concerned in the formation of the gelatine slime coverings. (5) The protoplast is divided into a peripheral layer carrying the coloring matter and a central colorless portion. A colorless hyaloplasm layer usually surrounds the peripheral portion of the protoplast under the cell wall, and a similar layer usually separates it from the central body. (6) Many granules containing the coloring matter are packed into the peripheral protoplasm, giving it the appearance of a homogeneous layer, which, however, by the use of concentrated magnesium sulphate solution and Zeiss' apochromatic lenses, may be easily resolved into its constituent granules. These granules contain the chlorophyll and phycocyanin blended together in one and the same coloring body, so that we have an association of coloring matter similar to that found in the Rhodophyceæ (54). (7) These granules which carry the coloring matter are regarded as the chromatophores and are therefore designated as "Cyanoplasts." They are connected by fine protoplasmic threads with the peripheral protoplasm and with a pocket-like layer of protoplasm surrounding the nucleus similar to the pellicle surrounding the nucleus of *Spirogyra*. (8) Starch or any starch-like substance is lacking in the Cyanophyceæ, but glycogen is present, which is the first assimilation product of these plants, because it appears and disappears according as the plant is grown in light or darkness. (9) Beside the cyanoplasts, the peripheral cytoplasm contains two other enclosures, the albuminous crystalloids (cyanophycin granules of other writers), and the slime vacuoles (slime balls of other authors). These enclosures are always found in the peripheral protoplasm of the cell. (10) The albuminous crystalloids are usually crowded into the heterocysts, being at the ends where the pore enters, or in the spores, or in the older vegetative cells that have ceased to divide. They are entirely absent from the young, rapidly dividing cells. Their crystalloid character is attested by their sharp angular outlines. Their albuminous character is shown by their chemical and staining properties. (11) In cultures grown in the dark, these albuminous crystalloids are consumed. They are also consumed in the germination of spores. Such facts, together with their always being formed where food material is accumulating caused Hegler to regard them as reserve materials. (12) The slime vacuoles are difficult to distinguish from the albuminous crystalloids except by staining reactions, and their composition cannot be exactly identified. Their staining properties, however, caused Hegler to consider them to be a slime material, very much like albumen, similar to that present in *Fucus vesiculosus,* and many other plants. It approaches nearest to mucin. (13) The chemical and physical properties of the granular parts enclosed in the peripheral protoplasm, together with their incapacity to increase by division, and their entire absence in the dividing cells indicate that neither of these granules can be considered as of a nuclear nature. (14) The principal point in the question of the Cyanophycean cell was that of the nature and importance of the so-called central colorless body. Hegler considered that his investigations had conclusively proven that these central bodies were the cell nuclei of the Cyanophyceæ, and that their behavior during division gave sufficient grounds on which to base this judgment. (15) In all cells of the Cyanophyceæ with the exception of the heterocysts where the nucleus early degenerated, there was a single cell nucleus whose form depended to a great degree on the size of the cell. In round cells it was spherical, in elongated cells elongated, the long diameters of the cell and nucleus being parallel. (16) The resting nucleus consisted of a slightly stainable foundation mass, in which were loosely imbedded small granules, each of which stained deeply with the ordinary (basic) nuclear stains after having been properly fixed with sulphuric acid. These granules, from their behavior during division and towards stains and chemical reagents, were identical with the chromatin substance of the cell nuclei of higher plants and animals, and on this account were designated "chromatin granules." They had no relation to the peripherally lying albuminous crystalloids or to the slime vacuoles, and they never appeared isolated in the peripheral protoplasm. They were therefore neither identical with the slime balls of Palla nor with the "red granules" of Bütschli. (17) The nuclei of the Cyanophyceæ differed from the nuclei of the higher organisms by their lack of nucleoli and the absence of any nuclear membrane. A sharp differentiation of the nucleus could be obtained by fixing with sulphuric acid and staining with Heidenhein's iron-alum hæmatoxylin. (18) During division of the cell, the small chromatin granules merged into one another and formed larger masses whose chromosome nature could easily be demonstrated from their behavior during division. Their identity could also be traced into the daughter nuclei. (19) As the chromosomes drew apart and took up their positions at the poles of the dividing spindle, there could, in every case, be made out a zone of delicate non-granular fibers (striated zone) connecting the daughter nuclei. These represented the central fibers of the achromatic spindle of higher plants. (20) The nuclear division was carried on entirely independent of the cell-plate formation, and was completed, or nearly so, before it had begun to grow in as a collar-like ingrowth from the equator of the mother cell. The ingrowing cell-plate slowly advanced until it forced the fibers of the spindle together into a thin thread or cord which pierced the cell-plate in the centre. This achromatic thread was finally severed, but the cells remained connected by a pore from one cell lumen to the other. (21) The polar movements of the chromatin substance and the formation of a chromatic figure in the case of the Cyanophyceæ, harmonized with the mitotic process of division of the ordinary plant and animal cell nucleus, so that he could not have any doubt of the nuclear nature of the forms hitherto known as the central bodies, in spite of the lack of a nuclear membrane and nucleoli. (22) In the division of the cyanophycean cell the nucleus was always divided into equal halves, and therefore could not be due to direct division or fragmentation, for in none of the places where fragmentation occurred in the higher plants did this occur. Again in fragmentation the division wall did not appear, while in the Cyanophyceæ it invariably followed the division of the nucleus. It therefore was settled that a nucleus was present in the Cyanophyceæ, and that it divided according to the usual mitotic methods.

It is to be regretted that Hegler did not leave drawings to supplement the very excellent photo-micrographs which illustrate the paper. Photo-micrographs, especially when unretouched, are always true to nature, but there must be a great advance in the art of making them before they can be made to show the details of cytology that good camera lucida drawings reveal. They form a good adjunct to the illustration of such a paper, but should not be relied upon entirely.

(2) GENERAL MORPHOLOGY OF TYPES STUDIED.

As has been shown by the foregoing review of the literature, the cell of the Protophyceæ is usually described as a protoplast composed of a central body and a peripheral part surrounded by a wall. This is as far as agreement goes. The composition of the central body, and whether it should be regarded as a nucleus, are points round which contentions have raged strongest. Most observers agree that the protoplast is granular, though some maintain that it is homogeneous. These granules are variously spoken of as chromatin, reserve products, assimilation products, etc. The peripheral zone is looked upon as a chromatophore by some, while others consider it as the general cytoplasm, throughout which the coloring matter is diffused. The wall is composed of a substance unnamed as yet, but supposed to be a modification of cellulose, probably quite close to fungus-cellulose. It was in the hope of adding something to our knowledge of these organisms, which might aid in the solution of these cytological problems that the present work was undertaken.

Material Used.

The choice of organisms for this investigation was governed by several considerations: (1) The availability of material. The greenhouses and Botanical Garden of the University of Pennsylvania have furnished excellent places for the collection and culture of these organisms, though the collections were by no means confined to these localities. (2) Plants were selected primarily with a view to the large size of their cells. Those forms in which the cell contents were especially masked for any reason, or where the cells were very small, were discarded, and the larger and clearer organisms used in preference. (3) Several different organisms were employed in order to avoid the mistaking of artifacts for natural conditions, and to eliminate the "individual factor" as much as possible.

Though the work here reported deals mainly with the species of *Oscillaria, Nostoc* and *Cylindrospermum* investigated, the results with the other organisms were essentially the same, and to speak of each organism in detail would be but reiteration. Therefore the morphological structures have been made the basis of report, they being so constant, varying only with the forms of the cells of the different plants, that they may be considered as belonging to all of the forms except where otherwise noted. The plants studied were as follows: *Nostoc* (three species), *Nostoc* in *Collema,* *Gloeocapsa polydermatica, Oscillaria imperator, Oscillaria Froehlichii, Oscillaria nigra, Cylindrospermum macrospermum, Spermosira litorea, Anabaena flos aquae, Tolypothrix lanata, Rivularia pisum, Gloeotrichia* and *Spirulina.*

Methods Employed.

One of the chief things to be determined in the study of the Cyanophyceæ is the best method of preparation of the material. In fact this is as important as the selection of the material itself. The Cyanophyceæ are amongst the most delicate of organisms that one can find in their reaction to environmental conditions, regardless of the varying surroundings under which different species will grow. For example, if organisms which ordinarily grow upon a moist substratum be subjected to increased moisture, the granular contents of the cells are modified or changed altogether. It is, therefore, absolutely necessary that in the cultures kept in the laboratories for study, the conditions of nature be simulated more closely than with any other of the algæ. This is no easy task, and many times in the midst of an interesting investigation, the culture would die and leave much of the accomplished work of little value. Not a little of the confusion in the interpretation of the cell contents of the Cyanophyceæ is due to a misappreciation of this point, as is evidenced by the methods of culture described in the various papers upon these organisms. Another point to be noted is the time of year when experiments are performed. A large part of the material used in the present investigation was found in various places in the greenhouses of the University, but even here, where the conditions seemed to be ideal, and where the requisites for normal growth were fairly constant through the whole year, the organisms showed a remarkable tendency to grow in cycles. Beginning at a certain period of the spring, they vegetated very rapidly and at such times dividing cells would be abundant. Gradually this growth ceased and the plants remained stationary for a season. Then a gradual decline began and no visible trace of the organism would be left. In this way the spores that had been formed lay dormant until the proper season of the following year, and no experimental method seemed to cause them to germinate sooner.

In the study of the organisms, it was the general plan to do as much on the living plants as possible. It was often found difficult to keep the trichomes in natural surroundings, *e. g.*, in the forms found creeping over the ground often the mere mounting in water would bring about extreme variation conditions. The organisms were studied carefully in their normal conditions under various powers of the microscope, the work being performed with Zeiss' achromatic and apochromatic objectives of 2 mm. and 1.5 mm. focal lengths, and compensating oculars 4, 6, 8 and 18. All of the drawings were made with the camera lucida and these objectives. Culturing in various nutrient fluids which would bring about accentuated or pathological conditions, was found to be an extremely useful mode of experimentation. Cultures of a full nutrient solution were found to yield particularly beautiful results; also fluids which would either feed or starve the chromatin were extremely useful. Growing in direct and in diffuse sunlight, under different colored screens, and in darkness were also tried. Plants were grown in weak solutions of different salts, as palladious and platinum chlorides, to assist in bringing out the structure in the cell wall, as recommended by Dr. Pennington (62). The organisms were also cultivated under natural and artificial conditions in a live cell and watched continually for long periods of time. In this way the same cell could be observed in different stages of its life-history. Intra-vitam staining gave very interesting results, especially when carried out on the life-slide and continuously watched. By this method chromatic and nuclear elements were very nicely contrasted. Filaments of *Spirogyra* were also placed among the trichomes of Cyanophyceæ to show the effect of the stain upon their nuclei. In every instance the nuclei of *Spirogyra* and the central bodies of the lower plants would give identical results. After as much was made out from the living preparations as possible, fixed and stained material were resorted to for the purpose of checking and supplementing the former observations. Any point thus noted would be again sought and found if possible in the living unchanged cell.

But as is well known, fixed material is open to the criticism that artifacts may at times be formed. To overcome this it was thought that if several different fixatives supplemented by several different stains should each bring out the same features constantly, the strong presumption would be that the structures shown would be natural to the organism, for in such a concert of results, obtained from such different chemicals and fixing methods, there would have to be some natural structure to act as a determining cause or there would certainly be differences shown in the fixed material. All of the experiments here detailed, and the results given, were thus confirmed.

The Cyanophyceæ have usually been quite scientifically investigated, but in the study of the bacteria, where the cells are so much smaller and probably more delicate, some methods have been employed which would not be countenanced at all in higher forms, and even such methods have, at times, crept into the investigations of the Cyanophyceæ.

The fixing reagents chosen were chromic acid of various strengths, chromacetic acid, corrosive sublimate—both hot and cold—picric acid in alcohol and in water, picro-sulphuric acid, osmic acid, Flemming's strong and weak fluids, Hermann's fluid, acetic acid of various strengths, formalin, alcohol, formalin and alcohol mixed, and boiling in water. Staining was effected with Heidenhein's iron-ammonia-alum hæmatoxylin, Delafield's hæmatoxylin, acid hæmatoxylin, saffranin, eosin, erythrosin, methyl green, methyl blue (for intra-vitam staining), methyl violet, gentian violet, picro-carmine, borax carmine and carbol fuchsin with the use of bacteriological methods of mordanting. Of these methods, fixing with Hermann's fluid, chromacetic acid or picro-sulphuric acid seemed to give the best results, and were the principal ones used, though all fixatives were used in determining each point. It is no easy matter to determine what fixatives to use and what stains to follow them with. One stain will work well with one fixative, or after a certain kind of treatment, while it will absolutely refuse to stain at all with some other fixative. This fact has seemingly been another cause for much confusion in the past. Alcohol was largely used for fixing the materials for micro-chemical experiments. The stains that gave the best and most constant results were iron-ammonia-alum hæmatoxylin, counter-stained with eosin or erythrosin, Delafield's hæmatoxylin, intra-vitam staining with methyl blue, carbol fuchsin, eosin and safranin with gentian violet.

In fixing and staining the organisms, a method that might be termed "*en masse* fixing or staining" was followed. A considerable mass of the organism was taken from the original culture and placed immediately in the fixing fluid, where it was allowed to remain the required length of time. It was then transferred "*en masse*" to water where any adhering dirt particles and the fixing fluids were washed away. When thoroughly washed the mass was placed in the staining fluids, or, if sections were to be cut, imbedded in paraffin in the usual manner and sections cut from one to two microns in thickness. However, it must be remarked that sections revealed very little that could not be seen equally as well in the uncut object. A difficulty arose from shrinkage of the cell contents in the imbedding process, which could only be overcome by very gradually passing the organisms from one strength of alcohol to another, the grades not exceeding 7 per cent. to 10 per cent. at a time. Special care had to be taken in passing from absolute alcohol to oil of cedar, else artifacts were sure to form.

Ordinary preparations were made by taking the unstained or stained organisms and carefully spreading them out on a slide in water or whatever medium was to be used for the examination. For filamentous forms, such as *Oscillaria,* this could easily be done by dropping the mounting medium upon the organisms on the slide, thus the individual trichomes would be separated, while, if in a pellet of jelly as in the case of *Nostoc,* the gelatinous mass was placed on a slide and carefully pressed out in the selected medium, when the trichomes would be plainly shown. In mounting, several methods were followed. Two per cent. acetic acid gave very good results and permitted the finest structures to be brought out. This acid also swelled some parts slightly and made some structures more visible though obscuring others. Another method was to mount in weak glycerin. This also gave satisfaction, but probably the finest results were secured by placing the stained plants in a 10 per cent. solution of glycerin in water on a slide without a cover and permitting the water to gradually evaporate until the glycerin was concentrated. Then after wiping away as much of the glycerin as possible, by placing a drop of glycerin-jelly that is just fluid on the specimen and covering, fine plump preparations were obtained. In these the minutest details were revealed without any apparent distortion.

In studying the Cyanophyceæ, it has proven of great value to supplement the observations made in the usual manner by numerous micro-chemical investigations into their composition. It is largely due to a failure to recognize the micro-chemical differences in higher plants that some investigators of the Cyanophyceæ have introduced so much confusion into the cytology of these forms.

The Cell Wall and Sheath.

The cell wall in its younger stages gives the characteristic reactions for cellulose with chlor-iodide of zinc and with iodine and sulphuric acid. As it grows older it loses this property, apparently from its being changed, or impregnated by some substance which is negative to all cellulose tests. It seems to be allied to fungus-cellulose, though Macchiati (50) considered it and the sheath to be the same as the cuticle of the higher plants. Correns (18) was able to get a cuticular reaction for it with an alcoholic solution of chlorophyll. The present investigation gave no reaction sufficiently marked to warrant the substance being termed cuticle, though the two may have close affinities.

The walls are laid down in distinct lamellæ by the addition of microsomata of cellulose upon their inner faces. Ambronn (1) found that there was a substance between the lamina of cuticular sheaths which disappeared upon boiling and reappeared when the plant was cooled. He, therefore, concluded that the substance had melted. Hegler (38) found that the laminæ retained their identity when heated in glycerin until the boiling point of the fluid was reached, which is much higher than that of water. In such forms as *Nostoc, Anabaena,* etc., all of the lamellæ, except the one immediately enclosing the protoplast and possibly at times a second, swell up and become a thick gelatinous protective zone. If a little care be taken in crushing out the colonies of *Nostoc,* this zone can easily be traced to consist of the successive generations of lamellæ, thus the inner unswollen wall which was formed last invests but a single cell. The one immediately outside it invests two cells, while the others in order will invest respectively four, eight and sixteen cells each. When these swollen sheaths are knotted up together they make the apparently homogeneous jelly of the *Nostoc* colony.

In some forms, as *Lyngbya,* the outer sheaths do not gelatinize, but remain tough and thin. If *Lyngbya* be cultivated in a weak solution of palladious chloride (1 :100,000) or in platinum chloride of the same strength, this laminated structure is beautifully demonstrated, and the laminæ are shown to be made up of cell walls surrounding groups of cells as in the forms where the sheath swells into a jelly.

In *Oscillaria* this sheath gelatinizes and is mostly dissolved away by the water in which it lives, leaving only the inner or last-formed walls directly investing the protoplasts. In younger trichomes, or those which have not been greatly disturbed, the sheath may be still more or less evident. It has a very faint tint after the action of eosin and other protoplasmic stains. When not completely dissolved away by the water, it may be demonstrated after mordanting with glacial acetic acid, and staining with carbol fuchsin or methyl blue. It is this stainability of the gelatinized portion of the outer wall, or what may remain of it, that has led observers in the past to think that a protoplasmic pellicle existed as a sheath around the outer wall of the trichome and caused a peristaltic motion in the organism. If such organisms be stained with acid hæmatoxylin, this thin coat of gelatin will take a deep reddish blue color, which will also be the result upon the gelatinous envelope if *Nostoc* or other such form be similarly treated, while the undoubted protoplasmic cell contents are stained quite differently. *Cylindrospermum* is encased in a protective layer of jelly-like consistency. The layer may be very thin, through partial or complete solution as in *Oscillaria,* or it may be thick enough to completely cover the ciliary processes (Fig. 74). This gelatinous covering is often "heaped up" around the spores and heterocysts (Fig. 56) where it sometimes frays out in irregular finger-like processes. It sometimes is weakly stained by eosin, but the color immediately disappears when the object is immersed in water.

By carefully manipulating the source and direction of the light, various markings may be found on the walls of the Oscillariaceæ. In some, definite cross lines are easily seen, averaging from five to seven striæ to each cell (Fig. 41), while in others the lines run longitudinally (Fig. 42). In optical section, the walls of the cells of *Oscillaria* show fine pores on the sides where cilia pass through. The same is true of *Cylindrospermum* and such other forms as possess these organs. They are best seen after corrosion with iodine and sulphuric acid (Figs. 27 and 63) when the wall becomes swollen and the pores become more evident. In the cells figured, the sulphuric acid has so plasmolized the protoplast as to cause it to withdraw the protoplasmic processes from the pores, leaving them empty and distinct, while the protoplasmic fingers which passed through them are shown projecting from the protoplast. In cross sections made with the microtome, these pores are quite evident, especially if the organism has been cultivated for a few days in a dilute solution of palladious chloride.

The Central Body.

The protoplast of the Cyanophycean cell is definitely divided into two portions, the central body and the outer protoplasmic zone. This distinction can usually be determined in the living cell. The central body in living material is generally quite colorless and filled with large grains or "slime balls" as they have been termed by some investigators. These balls may be so large as to give the appearance of a fragmented central body (Fig. 37). From their position and reaction, these are evidently what Bütschli (9) termed the "red granules," on account of their staining a reddish blue with Delafield's hæmatoxylin. Bütschli considered them to be composed of chromatin. Palla and Stockmeyer could not find them within the central body, but upon the outside of it, the reactions were such, however, as to lead them to conclude that they were composed of chromatin. When colored by the ordinary nuclear staining methods and stains, they are shown to have a periphery composed of a substance reacting exactly like chromatin, while the central part does not seem to take up the stain at all or very slightly. The central body therefore appears as if composed of a number of hollow chromatin vesicles. The fact that these vesicles are often quite large, especially in plants that have been cultivated in solutions which feed the chromatin, had led some observers to consider that they were vacuolated. But their behavior during division excludes the possibility of such an assumption. The central body is of no definite form, its outline being determined by the number of balls within it. In *Oscillaria* the central body fills up a large portion of the width of the cell and in some forms quite as much of the length. In *Cylindrospermum* the central body is more elongated in the longitudinal axis of the trichome, often reaching from end wall to end wall. By cultivating the organism in a full culture solution for several days, or in a solution strong in soluble phosphates and iron which feed the chromatin, the outer stainable walls of the "chromatin vesicles" become much more pronounced, while if cultivated in fluids which are poor in these substances and which would thus starve the chromatin they become degenerated, losing their power to take up chromatin stains. This is quite in accord with the work of Brass (6A) who was able in this way to so starve the chromatin of the nuclei of Amoeba and Gregarinida as to make them quite poor in this element. Digestion of these organisms in artificial gastric juice made the central body much more evident, though it dissolved away the surrounding protoplasm and caused the chromatin vesicles to take a characteristic yellow luster. Experiments upon *Spirogyra,* carried on in the same culture dishes with the above, gave identically the same results as regards the chromatin. When cultivated in a full nutrient solution, the nucleus became much denser in its staining properties and somewhat enlarged, while when the cultivation was carried on in a solution free from phosphorus the opposite was sure to occur. The central body, of the forms of *Nostoc* that grow in *Collema,* or of *Anabaena* found in the roots of *Cycas,* is very much poorer in chromatin than is that of similar plants growing under their natural environments. Bütschli found that when the Cyanophyceæ were digested in artificial gastric juice the central body floated free in the otherwise empty cell and showed a Brownian motion. The central body of the resting Cyanophycean cell is therefore usually composed of larger or smaller hollow vesicles of chromatin, as claimed by Lauterborn (47) for Diatoms. These vesicles are imbedded in a finely granular ground substance, which sends radiating lines toward the periphery, piercing the chromatophore. If a cell wall be ruptured and the cell contents pressed out, these hollow chromatin vesicles may be seen lying isolated within the protoplasm (Fig. 24). In cross sections the vesicles are shown to be quite numerous (Fig. 31) and of a small size in the naturally grown plant, but in material grown in the full culture solution they are relatively large and few in number (Fig. 30). Radiating from the central body are seen fine granular kinoplasm-like processes of the ground substance, which pierce the chromatophore and pass out to the cell wall where they form the central portion of protoplasmic ciliary-like growths, about one-half to one micron in length on the sides of the organism. In *Oscillaria* there may be as many as four such processes shown in optical section on one side of the cell, while on the other side only one or two may be seen. In *Cylindrospermum* these ciliary processes are more numerous and regularly distributed.

The central body has no membrane surrounding it. In this it is different from the nucleus of higher plants. Hieronymus has suggested that this condition be termed an "open nucleus." Palla, Strasburger and Fischer described a delicate membrane surrounding the central body, but in this investigation, nothing of the kind has been found. The opinions of various investigators of the Cyanophyceæ do not agree as to the nuclear nature of the central body. Zukal did not consider it to be a nucleus, but attributed that function to the cyanophycin granules, while he regarded the central body as the cytoplasm. Hieronymus considered it to be a nucleus, but without a nuclear membrane. Zacharias based his argument against the nuclear nature of the central body upon the fact that the chromatin did not increase during division. This can be accounted for by the fact that there is no nucleolus present, which in the higher plants contains the chromatin in a changed form during the resting stage, but gives it out to the spireme during the process of division. If, on the other hand, the chromatin vesicles should be termed nucleoli they retain the chromatin in an unchanged form, and therefore there is no apparent increase in the amount of chromatin at the time of division as in the higher forms. The apparent decrease in the amount of nuclein at the time of division mentioned by Bütschli may be accounted for by the fact of its becoming diffuse before forming the network, thus appearing to decrease, though in reality not doing so. Any hyper- or hypo-chromatin stages appearing in the processes of division, except those which come from the passing out of the chromatin of the nucleolus to the spireme and subsequently storing it up again, are probably to be looked upon as pathological. Zacharias also argued that the central body might be changed by environment, though this had never been observed for the nuclei of higher cells. Brass was, however, able to bring about some very profound changes in nuclei of Infusoria by varying their conditions of growth. The morphological changes spoken of by Zacharias are, however, really the changes due to the different stages of division, which, in the Cyanophyceæ, present very different aspects. Zacharias, in his reply to Bütschli, conceded the central body to be the starting point of the nucleus, and that its functions might be the same as those of the nuclei, but he did not consider it to be that organ. His conceptions of what constitute the essentials of a nucleus do not seem to be plain from his writings.

Palla considered the central body to be homogeneous, having no chromatin network, nucleoli or granules. Because of this lack and its division by direct methods, he concluded that the central body was not likely a nucleus, though it might have had a common origin with that organ. Hegler, on the other hand, was able to find all stages of mitotic division in the central body and strongly affirmed its nuclear nature. Scott and Dangeard also recorded the mitotic division of the central body and its consequent nuclear character. Chodat and Malinesco did not consider the central body to be a nucleus, and later Chodat, publishing conjointly with Short, described the central body as a vacuolated portion of the cell contents, laden with slime balls.

Thus the views concerning the central body of the Cyanophyceæ differed. Some attributed to it a nuclear nature with mitotic divisions. Others considered it a nucleus, but with direct divisions. Still others regarded it as not a nucleus, but the phylogenetic progenitor of one, or that it originated from a common organ with the nucleus of higher plants though itself not representing that organ, while some looked upon the central body of these organisms as in no sense a nucleus or even functioning as such, even calling it a collection of vacuoles or, in one case, the cytoplasm. Whether the central body should be termed a cell nucleus depends entirely upon our definition of that organ. If our conception of nucleus is that it must have all the attributes of nuclear membrane, reticulum and nuclear sap, nucleolus, etc., as in the higher plants, together with a definite form, then this central body does not fulfill the requirements of the definition. But if we consider a nucleus to be a centre in which is located the hereditary material of the plant, and which governs the constructive activities of the cells, such as assimilation, growth and repair, also reproduction of form or structure, if in other words, we consider the nucleus from the physiologico-morphological side, then there can be no reason for denying the nuclear nature of the central body. Even measured by the first requirement above mentioned it would not fall far short of the restricted conception of a nucleus. It has a much more definite form than the diffused nucleus of the Infusorian *Trachelocerca,* as described by Gruber (29), and the ground substance of the central body quite represents the more fluid parts of the nucleus, while Hieronymus, Strasburger and others have been able to find a definite nuclear membrane. The second, and more comprehensive definition of a nucleus, is certainly fulfilled by the central body of the Cyanophyceæ. There is as much evidence for the chromatin of the Cyanophyceæ being the hereditary material of these organisms, as there is to consider it such in the higher plants. Moreover, that the constructive metabolism is governed by the central body is evidenced by the fact that plants which have been cultivated in solutions which starve the chromatin, are never healthy and strong, the cyanophycin grains often disappearing. The definite threads of kinoplasm which radiate from the central body and pass into the ciliary processes, suggest evidence that the movements are directed by the same organ, while its activities in the formation of spores and in division show that it has much to do with reproduction. The argument that the central body has no stable morphology has been refuted by Bütschli (9), who showed that it is no more variable than true nuclei. Strasburger also mentions the finding of cells, no longer capable of division, which might have several nuclei, the result of fragmentation. The central body of the bacteria was studied by Nadson, who concluded that their nucleus was diffused throughout the whole of the cytoplasm, very much as the present investigation has shown the coloring matter of the Cyanophyceæ to be. This would mean the same intermingling of the functions of cytoplasm and nucleus as exists between the functions of cytoplasm and chromatophore in the Cyanophyceæ. This form of protoplast Nadson termed an "Archiplast." He often found scattered granules of chromatin in the Archiplast. Bütschli considered the bacteria to consist of a central body only, which often showed clear ends. Fischer suggested that this central body was merely a plasmolized protoplast, and that there really was no central body or nucleus to be seen in the bacteria.

If we are to accept the doctrine of evolution as a working hypothesis, these so-called "non-nucleated" organisms probably represent the progenitors of the higher plants, or a line of degeneration from them, or their nuclei, on account of the evident differences between them and the nuclei of other plants, may be the carrying out of a different line of development, though still developing a structure which fulfills the functions of a nucleus. Whichever case it should be, makes very little difference in our present argument. If, as has been largely accepted hitherto, these plants have no nuclei and still exhibit hereditary traits, it would seem to negative the whole theory of heredity as explained by mitotic division. One can hardly believe that the hereditary material would be placed in one structure in the higher plants and in another in the lower, and still be carrying out a line of gradual development, even though evolved along different lines of developmental history. In all other organs, homologies are carried out, and we should expect that if the hereditary material is located in the chromatin in higher plants, it is likely to be found in a similar substance in the lower ones. Accepting the doctrine of gradual development, to what are we to look for the antecedents of the nucleus? It is not probable that it came into existence with all of its mitotic steps, suddenly and *de novo*. All modern biological research would oppose such a conception. It would seem, then, that we must look for the beginnings of the nucleus in these forms which we have denominated the "non-nucleated" organisms, though, of course, through all the time passed since they first came into existence, modifications most likely have occurred and we would scarcely expect to find them just as they were in their early history.

In the higher plants the nucleus is looked upon as the controlling centre of cell activity (86). What, then, if they have no nucleus, can act as the governing agent in these lower plants? Strasburger's experiments seem to prove that proteids are formed only by the nucleus. If the Cyanophyceæ have no nucleus, what, we may say, forms the proteids which are demonstrable in their cell contents? These considerations would lead, *a priori,* to the conviction that there must be a nucleus present in these so-called non-nucleated organisms, or at least something that performs the functions of one, if we are to accept the great bulk of work done on many lines by cytologists. Whether this controlling structure in the Cyanophyceæ should be called a nucleus, or by some other name, the organ functions as such, and the difference is, evidently, a matter of the definition of nucleus, and therefore a mere matter of words. The localization of this controlling influence seems to be the problem. If it should be found that here we have cells exhibiting all of the properties of nutrition, growth, reproduction, heredity, etc., but devoid of chromatin in any form, it would seem to weaken the conception of the nucleus above referred to. But the investigations reported here show that the Cyanophyceæ are in no sense an exception to the scheme of evolution, but in reality one of the earliest steps in the phylogeny of the nucleus.

The Chromatophore.

Fischer was able, with hydrofluoric acid, to digest away all of the protoplast except a hollow, barrel-shaped structure which contained the coloring matter. This he termed the chromatophore. He therefore considered the Cyanophyceæ to be without nuclei, but to have a color-bearing organ. Bütschli, on the other hand, was able to digest only the outer portion, while the central body was undigested, and he thus drew the conclusion that nucleus and chromatophore were both present.

Cohn (16) discussed at some length the coloring material of the Cyanophyceæ, but did not deal with the question of the chromatophore. He found a green coloring substance which was soluble in alcohol, and which he considered to be chlorophyll, and a blue pigment which he termed phycocyanin which was soluble in water. Naegeli had termed the light green pigment phycochrome, and when the bluish tint was present he called it phycocyan, this latter being synonymous with the phycocyan of Kutzing. Evidently therefore the phycocyan of Nageli was a compound of the two pigments mentioned by Cohn, and the name phycocyan should properly belong to the simpler substance as applied by Cohn. A third pigment, phycoxanthin, was met with by the latter observer in those forms of the Cyanophyceæ which have the purple color. This he found to be a modification of the phycocyan, differing very little from the blue variety. Hansgirg found that the pigments of the Cyanophyceæ had the power to elaborate food without the presence of light, but whether this was due to the phycocyan, he does not say. Certainly it cannot be due to the chlorophyll alone. It would be of interest to know if the recently discovered Roentgen rays, or any similar cause, could be active upon the chlorophyll when the phycocyan is present. Molisch (55) demonstrated that the phycocyan was a crystalline albuminous body, easily recognizable in plants fixed in a solution of cupric sulphate. Warming (80) considered that there was no chromatophore except possibly in a few forms such as *Glaucocystis* where it might be slightly developed. Zacharias could not determine its presence because he could not see a bounding membrane. Crato (19) found a sharply defined chromatophore, having an amoeboid-like form, and with thick stratification. Zukal (103) thought that a highly organized chromatophore was usually lacking, though he was able to determine a thin utricle-like layer of clear protoplasm between the colored crust and the cell wall. Nadson (57) considered the whole colored protoplasmic crust to function as a chromatophore, though not exactly deserving that name, while Hieronymus considered this crust not only to function as such, but to be rightly so called. Chodat was unable to distinguish between central body and crust layer and consequently, with Deinega, considered that no special color-bearing organ was present. Zukal defined the chromatophore as any "portion of protoplasm which contained the coloring pigment." In general this seems to be a proper definition, though in its broad sense it would include the whole of the cytoplasm. In the Cyanophyceæ which serve for the basis of this investigation, the two pigments, chlorophyll and phycocyan, are dissolved in the outer protoplasmic zone of the cell, coloring the greater portion of it. Surrounding this zone is a delicate colorless protoplasmic layer separating it from the cell wall. The colored zone has no definite structure other than that of the protoplasm and the cyanophycin granules which it contains. In the sense of Zukal's definition, then, the colored zone would rightfully be termed a chromatophore, and it certainly functions as such, though it also has to perform the functions of the cell cytoplasm as well. This is not to be wondered at, for in primitive organisms like these, one would expect to find an overlapping of functions which will later be differentiated and performed by separate organs.

Hegler (38) brought together in tabular form the views generally held concerning the form which the coloring matter takes. It is here reproduced with a few additions.

I.—Pigment completely diffused in the protoplasm; Naegeli, Schmitz, Zacharias and the text-books.

II.—Pigment embedded within the walls of a web structure of the crust layer; Palla and Bütschli.

III.—Pigment in net-like plates surrounding the central body; Deinega.

IV.—Green chlorophyll in grains, embedded in a colorless fibrillar system; according to Meyer, moreover, the grains correspond to the chlorophyll bodies of higher plants and the blue pigment diffused in the cell sap; Hieronymus and Meyer.

V.—The blue and green pigments both in the same very minute granules, which are themselves embedded in, and connected by, a protoplasmic thread with each other and with a hyaline-like layer of peripheral protoplasm on the outer side and with a protoplasmic pellicle, or pocket, surrounding the central bodies, much as the nucleus of *Spirogyra* is surrounded; Hegler.

In higher plants the chromatophores appear to be segmented off from the nucleus. They seem to require some nuclear constituent before they are able to perform their function. It is not improbable that there may be some nucleoplasm in the outer zone of the Cyanophyceæ which has not yet been aggregated into definite forms as in the higher plants, and its function, together with that of the coloring matter, not yet divorced from the functions of the cytoplasm. Indeed this would be suggested by the fact that the peripheral protoplasm retains a very diffused coloring with Heidenhein's iron-ammonia-alum hæmatoxylin and other nuclear stains. This quite strongly differentiates it from the delicate surrounding colorless layer of ectoplasm which lines the cell wall. Such diffused staining is increased or diminished according to the composition of the culture fluid, as explained below for the chromatin of the central body.

The Granules.

Besides the chromatin vesicles of the central body which have already been discussed, two other forms of granules may be found in the outer protoplasmic zone of the Cyanophyceæ. Of these, the most common are the cyanophycin granules which permeate the greater portion of the peripheral zone, especially the outer portion of it just under the thin ectoplasmic layer mentioned above. These granules are variable in number, being more numerous in plants which have been growing under conditions where abundant nutrition can be obtained, and diminishing in number as the nutrition of the plant decreases. In dividing cells they are least in evidence. They are irregular in form and size, being quite small and round to sub-angular in shape. They appear thus to be either reserve or assimilation food products, probably the former. They are faintly stainable with Delafield's hæmatoxylin, taking a blue tinge. When treated with weak hydrochloric acid (4 per cent.), or 1 per cent. sulphuric acid, they disappear entirely. Chloral hydrate solution also seems to dissolve them. These are probably the same granules that were termed "reserve granules" by Bütschli and Nadson, and what Palla and Borzi called cyanophycin. Hieronymus considered these cyanophycin granules to be composed of chromatin, while Zukal thought them to represent the nucleoli. Deinega called all granules an isomer of starch. They are much fewer in number in the cells of *Nostoc* enclosed in the thallus of *Collema,* where their symbiotic relations probably cause them to give up their surplus or reserve food to the fungus. Slime balls rarely occur in these cells. A second form of granule found in the cytoplasmic layer is what Schmitz termed "schleimkugeln." They are larger than the cyanophycin granules, and appear to be composed of a mucous substance of greater or less consistency, often verging upon the solid state. Chemically, they give the reactions of carbohydrate substances. When treated with 6 per cent. solution of potassium hydrate they often swell, similarly to the paramylum grains of *Euglena.* They stain blue with Delafield's hæmatoxylin and red with eosin. Their apparent carbohydrate nature somewhat militates against their being identical with the reserve granules of Bütschli, though it allies them with the "schleimkugeln" of Schmitz. Bütschli and Nadson were unable to stain their reserve granules with hæmatoxylin, but found that eosin gave them a deep red tint. Cyanophycin granules are not dissolved by dilute hydrochloric acid, but digestion with trypsin in an alkaline fluid causes them to disappear. They are usually scattered throughout the peripheral protoplasm, or aggregated at times along the division walls of the cells. Especially do they take the latter position in *Oscillaria.*

Vacuoles.

Vacuoles have been described in this group by different authors. Hieronymus and Palla declared that they were normal structures, while Gomont and Zacharias claimed equally strongly that they did not occur in active cells. Zukal considered that they appeared upon degeneration of the cell. This is the case in all of the organisms examined by the writer. Vacuoles, as normal structures, do not appear in any of the Cyanophyceæ. They can be made to appear by cultivating the organisms in darkness or in an unpropitious environment. When they appear as pathological conditions upon the breaking down of the cells, they have no tonoplast as described by De Vries (21B) and Went (83), but are merely globules of disintegrated cell substance enclosed in openings within the protoplasm. That they are formed by the disintegration of the protoplasm is plainly evidenced by the presence of oil and other similar products. The vacuoles described by some authors as occurring in the central body, sometimes even filling it completely, are in reality the larger or smaller hollow chromatin vesicles as described above.

Other Cell Constituents.

The chemical composition of the various parts of the protoplast has been spoken of in the different sections treating of the organs in detail. There are, however, some substances in the cells of the Cyanophyceæ, the presence or absence of which have certain bearings upon the interpretation of the other constituents, and it may be well to mention some of these. By micro-chemical methods it is found that glycogen occurs constantly in the cells of the Cyanophyceæ as claimed by Errera (24), and is probably the form in which much of the food is found. The granules and vesicles of the central body give evident reactions for iron and phosphorus. The vesicles are not dissolved by gastric digestion, but take a strong yellowish hue, like the nuclei of *Spirogyra* filaments when placed in the same fluids with the Cyanophyceæ. Tryptic digestion also fails to dissolve the central body, as does 10 per cent. potassium hydrate solution. The central body gives faint tests for plastin. Neither bichloride of potassium nor iron reveals tannin. In cells that have ceased to multiply, osmic acid reveals oil droplets in the outer protoplasmic zone. It will be seen, therefore, that these and other micro-chemical results that have been mentioned above, give strong confirmatory proofs of the conclusions here drawn.

(3) Morphology of the Dividing Cell.

The resting stage of the cell differs greatly from the dividing condition of the same cell. In the former it has been shown that the chromatin is aggregated into a number of hollow vesicles. As the cell begins to divide, these vesicles give up their chromatin which becomes very diffuse throughout the central body (Fig. 6), gradually forming into a more or less loose network (Figs. 2, 16, 67). This network is composed of faintly staining threads along which, and especially at the points of juncture of one thread with another, small chromatin granules are situated (Figs. 2, 16). These chromatin granules multiply by divisions that are transverse to the axis of the thread (Fig. 23). This division is not the longitudinal splitting found in the spiremes of higher cells for the purpose of equally dividing the probable hereditary material, but is apparently merely a multiplication of the number of granules upon the net. Thereafter two modes of division may occur, even in the same species. In the first method (Figs. 1 to 5, 16 to 20), the central body or nucleus does not get beyond the network stage. This network draws together along the equator of the cell (Fig. 3), the net becoming finer and denser. It then begins to constrict in the middle (Figs. 4, 7). The side walls grow inward, first as a delicate collar of microsomata, gradually becoming stronger and deeper, until finally the network is constricted entirely, and the microsomata harden into a division wall. In this mode of division, the nucleus becomes constricted in dumb-bell fashion. The halves are usually as nearly equal as can be determined by the microscope, but at times one will appear considerably larger than the other. Lauterborn (47b) has noted that the nucleus of Dinoflagellates divided in the spireme stage, though there the divisions and the spiremes were more typically mitotic than in the Cyanophyceæ.

The other method of division is undoubtedly a primitive mode of karyokinesis. The network is the same as has just been described for the first method of division, but instead of constricting itself into two parts while in the net stage, the chromatin network resolves itself into a single coiled linin thread (Figs. 8, 68), or spireme, upon which the balls of chromatin are arranged one against the other like a string of beads. This spireme arranges itself along the longitudinal axis of the cell, and breaks up into segments of about equal length (Figs. 9, 69). These segments might be termed chromosomes, though they do not form a nuclear plate, but are arranged along the whole of the segment in the form of the original beads of chromatin that were found in the net and spireme. That these beads cannot be termed chromosomes is evidenced by the fact that they usually retain their identity until after the daughter chromosomes are formed, each daughter chromosome being composed of several of them. The segments do not converge to the poles as in the spindles of higher plants, but each seems to end loosely in the cytoplasm. The spindle might, therefore, be termed "open," in the sense that for the time each chromosome appears to be independent. After the formation of the above spindle, the chromosomes divide in the middle, half of the chromatin retreating towards each end, but they retain the bead-like appearance for some time, and leave a linin thread connecting the two daughter chromosomes (Figs. 10 and 70). At this stage in the division there is formed, around the equator of the cell, a collar-like ingrowth of microsomata which gradually grows inward and constricts the linin connective threads (Fig. 71), which after they have been pressed to the centre, separate, but leave a pore connecting the two cells. Evidently the chromosomes are here divided in a transverse direction. The fact that they usually retain the bead-like structure until after they have separated into daughter chromosomes militates against the view that the chromatin has mixed, and that in this way a qualitative as well as quantitative division is effected as has been supposed to be necessary in the higher plants. The number of nuclear segments is not constant. There may be several (Fig. 9) rather fine chromosomes, or they may consolidate into two or more heavy segments (Fig. 11), often looking like heavy bars of chromatin without any beaded appearance. These heavier segments divide in the same way as the lighter ones, revealing the linin thread between the divided portions (Fig. 13). The ingrowing cell wall soon reaches the linin connectives between the separate portions of the segments of the chromosomes, constricting them (Fig. 13) and finally separating them entirely. The chromatin then becomes diffuse and later forms the vesicles or the network, according as the cell continues to divide or enters upon a period of rest. The ingrowing collar which finally forms the division wall, in the earlier stages is composed of very fine microsomata of cellulose. These gradually fuse to form the wall (Figs. 4, 5, 7 and

71), which divides the cell into two, always, however, leaving a small pore at or near the centre through which the protoplasm of one cell is connected with that of the next.

These observations, therefore, seem to show that two methods of division occur in the Cyanophyceæ. In one of these the nucleus does not pass beyond the net or spireme stage, which constricts itself into two nearly, though not necessarily equal parts. In the other method we have a very evident primitive state of karyokinesis, but no longitudinal splitting of the chromosomes, such as has been held to be necessary to equally distribute the hereditary materials in the higher plant cells. Whether there is any sequence to the occurrence of these different methods of division, so that one form might appear at one time in the life-history of the plant and the other at another cannot yet be asserted. There is no reason why such should not occur, however, for the Cyanophyceæ are evidently of a much more complex organization than we have hitherto supposed. But the fact that the two forms of division appear, and that each starts upon the way to karyokinetic division, one stopping in the spireme stage while the other goes on to the formation of a rudimentary spindle, is significant. The fact that the division of chromatin is not always equal and never longitudinal or qualitative, seems to suggest that this process is not so essential to the division of the hereditary material as has been held in the past, but that it has rather been evolved as a convenient method of giving out to each daughter cell the quantitative, rather than the qualitative amount of chromatin which belongs to it. This is further suggested by the method of division, which does not pass beyond the net-spireme stage. It is not in any sense a fragmentation of the nucleus, since it is invariably followed by the formation of a division wall. This is laid down in the same way as in the cells which show typical karyokinesis. It seems, therefore, that division in these forms is evolving towards a karyokinetic stage, but that in some cells it does not reach as advanced a condition as in others.

In those cells where the spireme segments or chromosomes are formed, each segment or chromosome seems to act independently, though all divide at the same time. When the daughter chromosomes separate, each retreats to its end of the spindle and there begins to diffuse, there being as many such centers of diffusion as there are chromosomes. These centres gradually merge together and form the granular nucleus out of which is to form the next net-spireme of the next division, or the chromatin vesicles of the resting stage.

(4) PROTOPLASMIC CONTINUITY.

The Cyanophyceæ have usually been looked upon as composed of groups of cells, but with no connected organization. As will be seen by the review of the literature, some investigators have been able to find pores in the division walls of these organisms, through which delicate strands of protoplasm passed connecting one protoplast with the other. To Borzi is due the credit of having developed this fact to the greatest extent. He found that the protoplasts were usually connected by means of these protoplasmic threads, but that the heterocysts, which showed the most evident openings were connected to the other cells by means of cyanophycin threads. This protoplasmic continuity between the cells, Borzi concluded, assisted the plant to correlate its movements. This is certainly a logical conclusion, and one that is supported by observation. The evident passage of material from which to form the spores, from cell to cell through these openings, is still stronger evidence of the concentrated organization of these plants. Macfarlane (51) considered that in the higher plants, sex-forming products might pass through these pores from cell to cell and thus correlate and epitomize the hereditary substance. Gardiner held that ferments and soluble materials passed through the protoplasmic threads in such of the higher organisms as exhibited them. The almost, if not quite universal, exhibition of intercellular protoplasmic continuity is most significant in this respect.

In treating the cells of the Cyanophyceæ with iodine and sulphuric acid, the partition walls are so swollen that the stained protoplasmic threads and the pores are much more evident. In this way every organism studied, was found to have intercellular protoplasmic continuity. When preparations are made from material killed in picric acid, their contents stain best to show this feature. In *Oscillaria* it is quite difficult to demonstrate such, but after the above treatment with iodine and sulphuric acid, where trichomes are broken across as seen in Fig. 25, the short protoplasmic connection stands out quite plainly. Some material which was collected and placed immediately in 95 per cent. alcohol, when examined was found to have plasmolized by the rapid withdrawal of water from the cells, so that the material was apparently worthless. But upon re-examining this material, it was discovered that every protoplast still held fast to the cell walls at certain points. Upon careful staining and examination, these points proved to be the places where each protoplast passed through the cell walls to connect with another protoplast, or to form a cilium upon the outer wall. This condition is shown in Fig. 26. At every point where one protoplast is seen apparently attached to a division wall, it will be observed that there is a corresponding attachment of the protoplast of the next cell. While examining some trichomes of *Oscillaria* that had been stained with iron-ammonia-alum hæmatoxylin, and that had slight pressure applied to the cover-slip, the end cell which bore the long finger-like hairs was seen to be pressed somewhat away from the other cells of the trichome (Figs. 43 and 44), and several fine protoplasmic threads were seen connecting it with the other cells. The central thread was always much heavier than the rest, which, though delicate, were still quite visible. In *Cylindrospermum* the division walls thicken a trifle at the points where the pore is formed (Fig. 74). Sometimes several pores are evident between these cells.

(5) THE HETEROCYST.

Heterocysts, like the spores, are modified vegetative cells. The pore, through which the protoplasmic strand passes to connect it with other cells, is usually quite large and the walls on each side are swollen into decided ridges. Macallum (49) thought the heterocyst a degenerated cell which might be the product of some rudimentary process. Hegler (38) called the cyanophycin granules "albuminous crystalloids" and considered that the heterocysts were crowded with these, especially at the ends where the pores enter. Borzi (6) also considered that the cyanophycin passed into the heterocysts through the pores at the ends. The development of the heterocyst of *Cylindrospermum* has been described above. In *Wollea saccata* and in *Nostoc* species in general the heterocyst is a cell somewhat larger than the vegetative cell. In earliest development the nucleus breaks up into a thick spireme as if about to divide (Fig. 75), but immediately disintegrates, becoming diffused as fine granules with here and there a few larger ones (Fig. 76). During this time there has been passing into the heterocyst from the other cells, through the pores, a substance which forms a deep staining end to the cell (Figs. 77 and 78), when it is stained with iron-ammonia-alum hæmatoxylin. This substance is gradually passed into the heterocyst from the adjoining cells on each side until the whole cell is gradually, but completely filled (Figs. 78 and 79). The substance is insoluble in dilute hydrochloric acid (2 per cent.) and by digestion in artificial gastric juice. With the latter treatment it assumes a golden hue similar to a cell nucleus. It would therefore seem to be composed of a substance related to chromatin, but this needs further investigation before it is affirmed as a fact. Macallum has shown that its substance also contains "masked" iron. This view would further lend color to its chromatin nature. What the function of the heterocyst may be, can only be a matter of conjecture at the present stage of our knowledge. The fact that the heterocyst is usually next or near to the spores might lead us to consider it as a storehouse of food for them. The fact that when spores and heterocysts are in connection, as in *Cylindrospermum,* they remain so long after the trichome is broken up, would also appear to substantiate this view.

(6) Spore Formation.

One of the striking features of the Cyanophyceæ is their ability to withstand long periods of drought. Plants will desiccate to such an extent that their size will not be more than one-half of their original dimensions or about one-eighth of their original bulk, and still if the proper environments be restored, the cells will revive and begin to vegetate and multiply. Such cells resume their activities in a strikingly short period of time, and frequently with very little moisture. In the rain-pools formed by the scanty rainfall on the deserts and semi-arid districts of the western part of the United States a decided coating of *Oscillaria* will frequently be formed within a few hours, all of which has developed from such desiccated cells, which, during the so-called rainy season, seem to be the principal means of tiding the plant over the periods from one rainfall to the next. These cells should not be termed spores, however, as their cell contents do not undergo the changes apparently necessary for the formation of a resting spore that will bridge over long periods of drought. Such desiccated cells, when collected by the writer in their native haunts, soon lost their vitality, not even resisting a five weeks' trip eastward to the laboratory, though it was easy enough to revive them three weeks after collection. The real spores will grow after a resting period of at least a year and a half, which is as long as I have had them under observation.

The formation of spores is a much more profound process than the mere drying of the vegetative cells, and it is probable that more than one cell, and possibly the whole plant, takes part in it. In *Oscillaria,* one to four cells will begin to enlarge to form a spore (Figs. 51 and 52). The adjoining cells to these gradually disintegrate and pass their chromatin elements into the forming spore, the turgor of which causes these cells to become concave (Fig. 52). The chromatin of the nuclei of the spore cells loses its vesicular appearance, and forms dense staining masses in the cytoplasm which gradually disappear owing to the diffusion of the chromatin throughout the whole cell. This diffused chromatin again aggregates towards the centre, forming there a granular chromatin body. If two or more cells are absorbed in the formation of the spore, as is usually the case, the original partition walls become absorbed and the protoplasts flow together, thus forming one spore with a large diffuse central body of chromatin. Whether this fusion should or should not be looked upon as a form of sexuality is problematical, however, for the reason that spores are sometimes formed in *Oscillaria* as in *Cylindrospermum* and other Cyanophyceæ, by the transformation of only one cell, but even here we find the same passing of chromatin into the single spore cell from the adjoining, or as they might be termed, the nurse cells. In higher plants, where undoubted sexuality occurs, it is nothing more than the passing of the substance of one cell into another, usually in the form of a definite body. In the Cyanophyceæ we find similar passage of a substance into the reproductive spores, but the substance is not here differentiated into a definitely formed structure. This should then be looked upon as the sexual act rather than the fusion of the whole cells as mentioned above. After the spore has formed, the protoplast lays down a dense wall which protects it throughout the resting period. The spore wall ruptures when the conditions favorable for growth are restored at the close of the resting period, and the protoplast pushes out, dividing as usual (Fig. 45).

Another method of multiplication by spore formation was observed upon one occasion. At the time in question, while preparing a life-slide for the continuous observation of *Oscillaria,* a trichome of unusual appearance came into the field of the microscope. The end, or cap, cell, from which the finger-like hairs were protruding, was in place (Fig. 47), and the third cell was perfectly normal, apparently having given up none of its chromatin as in spore formation. The cell between these two, however,—the second cell of the filament,—had every appearance of a spore with the exception of the heavy wall. The trichome was actively moving forward, which is not the case in plants that are forming, or have formed, spores in the manner described above, the cilia having been withdrawn and the end hairs absorbed. While observing this trichome, the end cell and the adjacent enlarged spore-like cell separated from the rest of the filament and moved away slowly by means of the slow crawling movement of the finger-like processes. These processes were finally absorbed (Fig. 46), and the spore-like cell began to divide, the dense, diffused, nucleus-like centre separating into halves (Fig. 46) and a dividing wall growing between them. Division continued until a six-celled stage was reached, by which time the cilia were formed and it could swim about freely in the water of the life-slide. On account of these movements it could no longer be kept under constant observation, but development had progressed far enough to show that it was forming into a new trichome.

In *Cylindrospermum* the process of spore-formation is somewhat different, but follows the same general lines. Usually the end cell of a filament which has no heterocyst becomes strongly chromatic, probably through the chromatin being passed into it through the communicating pore from the other cells of the trichome. This passage of chromatin is quite evident from the appearances of the cells and the behavior of the spores. The end cell then divides, and the sister cells thus formed grow apart about one quarter the distance of the short diameter of each cell, though both still retain a strong protoplasmic connection. Part of the jelly-like sheath of the trichome masses itself around these cells as they begin to grow; the short cilia that were found upon them when they were vegetative cells now elongate into strong hair-like flexible processes, passing from the protoplasts of both cells through the cell walls. The protoplasm of the outer or end cell becomes granular, and its chromatin diffused. It soon ceases to grow and becomes the heterocyst. The sister cell, being in contact with the other cells of the trichome, can draw upon them for nourishment through the intercellular pores. It grows greatly (Fig. 59), the chromatin remaining in the vesicles as in the vegetative cells, but here the cells are much more abundant and multiply by division until they fill nearly the whole lumen of the cell (Figs. 55, 57, 60). After the spore has grown to the full size, it withdraws its hair-like processes, forms a thick laminated wall around itself and becomes dark brown from the deposition of pigment (Figs. 61 and 62). The heterocyst later loses its hair-like appendages, and the organism settles down into the resting stage. The cells all disintegrate, except the resting spore and the heterocyst, which remain intact and connected for a long period of time, the heterocyst being filled with food substance in the form of cyanophycin. In *Cylindrospermum* the heterocyst cell is usually the terminal cell of a filament. It merely has the nutriment which was formed within itself, or that passed into it before it separated from the spore cell. But if the organism be grown in a full nutrient solution, or if the cell which divided to form the original spore and heterocyst be in the middle of the trichome as is sometimes, though not frequently, the case, then the heterocyst cell will develop into a spore also. The development of this spore into a trichome will depend upon the amount of material passed into it from the other cells. If it has formed in the middle of a trichome, and can be fed as the spore cell is, it will develop into a spore of as much strength as the other cell, or if it has only a few cells to feed it, it will be smaller and weaker. This would seem to argue that in *Cylindrospermum,* at least, the heterocyst is not a dead cell as it is usually supposed to be. All stages in its development, as here described, have been seen and it is not a difficult task to reproduce the condition. Not infrequently the terminal heterocyst, in material grown on a soil saturated with a full culture solution, will divide once before its chromatin becomes diffused, thus forming a double heterocyst (Fig. 56). After the formation of the spore, and the deposition of the plug which closes the pores at the ends of the spore cell, the chromatin which has been passing along the trichomes may not cease to flow, but become heaped up within the cell next to the spore, which then begins to grow and becomes a spore (Fig. 64) like the first, and with all of the steps taken by it except the preliminary division which cuts off the heterocyst.

In *Spermosira* the spores may form in any part of the trichome. One spore usually forms first in the manner already described for *Cylindrospermum,* except that the heterocyst cell is not cut off as in that organism. The chromatin here does not take the form of vesicles, but is in the form of more or less angular grains. After the formation of the first spore, others commonly form on either side of it, until a long string (Fig. 64) has been developed.

In *Wollea saccata,* the spore cell receives the material that is passed along the trichome just as in the other forms. It thus grows to twice or even three times the thickness of the vegetative cell and elongates until it is six to eight times the length (Figs. 84 to 86). The chromatin first becomes diffused, but gradually forms into large angular masses of irregular shape.

(7) MOTILITY.

The power of movement among the Cyanophyceæ is quite marked in some forms, and is practically the only distinguishing feature between some genera. In *Oscillaria,* the movements are most marked, and may be divided into three classes: (*a*) Creeping or forward movement of the whole trichome, by which it propels itself through the water in a serpentiform fashion. (*b*) An oscillating movement throughout the whole trichome, which gives the name to the genus. This movement might also be divided into two: (1) the general oscillation, and (2) the more rapid flexion of the extreme end of the trichome which usually closes the oscillation. (*c*) A spirally twisting movement which accompanies the oscillation. *Cylindrospermum* and several others of the Cyanophyceæ exhibit the first or creeping movement above mentioned, while the hormogonia of those forms which bear them are free-swimming, sometimes with a twisting motion as in *Nostoc* (77). Such movements among plants have been considerably studied, principally on Diatoms, and from these, generalizations have been made to include other organisms. Several theories are advanced to account for the phenomena, but most of them seem untenable. Pritchard (64) has given a very good review of all of the theories held by different authorities concerning the cause of the movements in diatoms, prior to 1861, therefore it will be unnecessary to more than glance at a few of them here and note their bearing upon the question of locomotion in the Cyanophyceæ.

Naegeli (58) explained the motion as caused by osmotic currents between the cells and the surrounding water. He says: "Since in the course of their process of nourishment they take up and secrete fluid stuffs, so the cell must come into motion when the attraction and expulsion of the fluids are unequal and so lively that the opposition of the water is overcome." He also speaks (59) of the power of *Oscillaria* to move independently of their sheath, attributing the movements to the same cause.

Max Schultz (70) supposed that the movement was due to protoplasmic creeping of the organism upon the surface of the slide by means of protoplasmic pseudopods passing through the cell walls, and continuous with a delicate contractile pellicle of protoplasm surrounding the cell, though he was scarcely able to demonstrate the pellicle. He was able to show the pseudopods by passing over them currents of water in which free coloring matter was ground up and suspended, the fine granules of color adhering to the protoplasmic processes. He believed that the plant could not move unless in contact with some object, as the slide or another trichome of the plant. He could easily make out the movements of particles of indigo on the trichomes of *Oscillaria* as described by von Siebold. These movements sometimes became rapid, and in the thicker species the particles at times revolved in a spiral. He noted that there was secreted a slimy substance which cemented the indigo particles together, for he could see the moving trichomes dragging the particles after them for a while, and later he could trace where a trichome had passed by means of the agglutinated particles of indigo which formed a kind of tube in its track. The cause of movement he considered to be entirely similar to that of Diatoms.

Von Siebold (72) wrote as follows: "The Oscillarieæ offer a very interesting sight when we notice their turning movements in water which has been colored with indigo. All of the pieces of indigo which come in contact with the Oscillarian trichome are passed into a slender spiral along the filaments towards the end, and the filaments may either remain stationary or move forward. It was surprising to me that sometimes these forward gliding motions of the indigo would take place on both sides of the trichome towards the middle where the coloring material is heaped up in balls, or that this movement would sometimes pass from the middle to both ends. There must take place, moreover, in *Oscillaria* an extensive secretion of slimy material, since the particles of indigo that are shoved together remain thus cemented in heaps for a long time." It will thus be seen that von Siebold considered that *Oscillaria* moved forward by means of endosmotic and exosmotic currents, the substance excreted being of a more or less slimy consistence, and helping to push the organism along. This is quite similar to H. L. Smith's (73) observations of granules streaming away from the frustules of Diatoms and propelling them forward quite like the means of locomotion of some parasitic infusoria.

Otto Müller (56) considered that protoplasmic currents passed through the raphe of the diatom cell wall, receiving thereby a spiral motion which caused them to act as a sort of propeller to the organism. In answering the claim of Müller, Lauterborn (46) strongly combated his views, claiming that the impinging of the spiral currents of protoplasm against the water would not be sufficient to cause the plant to move, and he would not base a theory of this kind upon theoretical grounds alone, which he claimed that Müller evidently did, for no one has ever seen protoplasm issuing from the raphe of diatoms. Müller was able to demonstrate that the plants could swim about when free from contact with any substratum.

The osmotic theory was defended by Kozlowski (43), who built up a very novel theory of phototaxis. He claimed that a difference in the amount of light influenced the two ends of a frustule. Thereby different rates of assimilation were set up, which caused the osmotic currents and motion. He based these observations upon the action of the organism towards the source of light as it came through the microscope. But unless he could demonstrate that there was no motion in the diatom in its natural surroundings, or that the intensity of the light would differ appreciably after passing a distance of half the length of a diatom, one can scarcely see the relation of his observations to organisms as they occur in nature.

Borzi (6) concluded that in general the mechanism of the movements of *Oscillaria* cannot be explained. "It only takes place," he said, "in this organism when the plant is not multiplying, and as soon as multiplication begins, the filaments come to rest, and the sheath becomes much thicker." He demonstrated a continuity of the protoplasm from cell to cell through a pore in the transverse wall, and this assisted the plant to correlate its movements. Neither isolated cells nor any form of the Cyanophyceæ that have heterocysts were capable of movement according to his observations. He was also led back by the different movements of *Oscillaria,* to accepting a helicoid movement of the filament due to heat, light, etc.

F. Cohn (15) considered that *Oscillaria* required a solid substratum to glide over. Without this they could not move. He based his conclusions upon their power to spread over the sides of the glass vessel in which they were being cultivated, and even to rise above the surface of the water. He never found *Oscillaria* filaments swimming freely in the water. They generally used other filaments as supports when they could not reach other substrata. "In *Beggiatoa,*" he said, "there are short waves of contraction which run over the filaments, and set them in a kind of peristaltic motion." These peristaltic contractions "are seen until they cease by the death of the filament." This is quite similar to what De Bary (20) has described for germinating *Cylindrospermum,* while Zukal (101) saw wrinkles on the sides of *Oscillaria* as it swayed backwards and forwards. Cohn then continued: "According to these observations it cannot be doubted that within *Beggiatoa* and the Oscillarieæ in general, there is a contractibility which is made manifest by alternate shortening and stretching of the opposite halves of the cells, and depending partly on the living energy of the cells and partly on the ductility and elasticity of their membranes." In another place (17) he carried out the same idea of expansion and contraction as follows: "Flexible *Oscillaria* filaments are capable of bending themselves spontaneously and of stretching themselves either straight out or curving themselves into snake-like forms, or entwining around other filaments." He could faintly make out a delicate protoplasmic pellicle covering the whole trichome. The unequal contraction of this pellicle assisted to cause the unequal contractions noted.

Hansgirg (35) concluded that motion was due to heat and light. The creeping movements took place within a very thin gelatinous sheath which fastened itself to some substratum and the organism moved backwards and forwards in this, leaving it extended behind the organism in a reed-like tube. This substantiated the observations of Schultze (70), though he considered the reed-like tube to be visible only on the sharpest inspection. This surrounding layer of slime was considered to be the same as that which Engelmann (22) described as a surrounding protoplasmic layer, though on account of its different stainability with iodine, Hansgirg concluded that it was not protoplasmic. Further, if the organism was cultivated in darkness, this sheath of slime disappeared and the organism lost its motility. He thought the force, which caused the various movements of *Oscillaria*, was generated by osmotic currents passing into the protoplasm of the filament. "For," he continued, "I have established by definite observations that a powerful strength of imbibition is peculiar to the protoplasmic content of the cells of this organism, and this strength of imbibition works as long as the cell lives." And again he said: "According to my view, the movements of those *Oscillaria* filaments which are enclosed in an osmotic sheath in which they move backward and forward are produced by diosmotic processes in consequence of which the turgor becomes greater in the cells of one end of the filament than in the cells of the opposite end. As long as the turgor remains in this one-sided condition, the filaments move forward in one direction, when, however, by an influence (*e. g.,* the friction of the foundation) the turgor of one of these cells grows less, that of the other end increases and the movement is reversed." Those specimens which grew on the surface of the soil or other substratum were always surrounded by a delicate pellicle of water in which they moved by means of these same osmotic currents. In addition to these currents he thought the contractility of the protoplasts of the cells also assisted in causing motion though this had not been shown directly.

Engelmann (22) saw in *Oscillaria* the inconceivably delicate slime pellicle of von Siebold and others surrounding the whole trichome, but interpreted it as a protoplasmic layer which by unequal contraction caused a peristaltic movement of expansion and contraction. This he thought was what caused the creeping movement of *Oscillaria.* When in contact with some supporting substance, inconceivably delicate as this protoplasmic envelope was, nevertheless it could raise an object of over one thousand times the weight of the trichome. He separated the oscillatory and spiral-like movements from the creeping motion. The former were almost exclusively seen when *Oscillaria* was free-swimming and did not need any supporting substance. He said: "Their absolute speed is much more definite than in any known case of protoplasmic motion. Without doubt they, the motions, are brought about as in the case of related bacteria, by glittering hairs, which are closely akin to whips. In some forms these flagella are seen. There is known no other source of similar expression of strength in the animal realm, and it is sufficient for a complete expression of all the facts."

It is regrettable that Engelmann has left no figures to demonstrate the position of the flagella described by him. The probability is that he referred to the long hair-like projections from the end cells, which have been described by Borzi and Hansgirg as parasitic organisms, and to which he here gave the functions of definite plant organs. He also intimated that pseudopodia about 0.03 mm. in length passed through the cell wall. The only figure in all of the literature reviewed which shows any such organs, other than these so-called parasitic growths, accompanies Zukal's paper (101) where he figures several long whip-like flagella springing from the division walls of *Cylindrospermum stagnale,* though no mention of them is made in the paper. (See Fig. 65, which is copied from Zukal's drawing.)

Hogg (42) found cilia on the ends of diatoms and in isolated positions along the sides, but his views have received no support from other investigators, and it is likely that he misinterpreted effects of lighting, as was pointed out by Wenham (82), who considered motion to be due to a protoplasmic layer surrounding the frustule.

Wolle's views (87) were expressed by him as follows: "By the careful observation of living plants the process of cell multiplication can be readily detected in the larger forms. Ordinarily the split of a cell commences on one side and then continues from the opposite side; a number of cells dividing at the same time will have the tendency to throw the end of the filament out of line, first on one side and then on the other, thus producing a vibratile motion. The process of creeping may be conceived in connection with growth, and yet it may not satisfactorily explain every movement. The apparent correspondence between the rapidity of growth and that of the creeping filaments is not without significance. The larger forms of *Oscillaria* are found to grow by cultivation at the rate of about one-half inch in an hour. The creeping of the same filaments progresses at the same rate, age of the filaments and other circumstances corresponding; hence the reasonable inference of relation between the two movements." Zukal (98) also regarded the motion of *Spirulina* as intimately associated with growth of the organism, and as comparable to the growth of a tendril.

Palmer (61) has observed in *Eunotia major,* protoplasmic processes at the corners of the frustules, which he called "coleopodia," which were connected with a thin layer of protoplasm or "coleoderm" surrounding the whole diatom. He said: "The above results of observations and experiments would seem to be conclusive proof that *E. major* and by inference other nearly related species and genera of the Fragillarieæ move by the action of organs that may be called coleopodia. This conclusion, however, is far from touching the question of the means of locomotion in *Pinnularia* and its allies. It may be added, in this connection, that while a large *Pinnularia* in rapid motion not infrequently gives evidence of brisk internal currents, such as Müller (56) has described, such currents have not been observed by me in *Eunotia.* Under rather high powers one only sees, near the corners of the frustules, in the vicinity of the raphe, a certain internal commotion among the very small protoplasmic granules, a spasmodic movement back and forth, a waving about. This movement differs alike from the streaming of cyclosis and the Brownian trembling, and it is traceable with difficulty, if at all, far from the corners. Nevertheless, the channelling of the frustules is of a character to indicate the existence of currents, and further observations may yet reveal them."

Barkas (2), in speaking of the movement of *Bacillaria cursoria,* observed that "the small, gritty particles on the sides of the frustules moved freely backwards and forwards, in a hurried manner along the edge of the frustules, as though they were occasionally driven by cilia, or as if they had automatic or voluntary motion. I observed that they moved when the diatom remained stationary."

It seems to me that the osmotic theory can hardly account for the movements of these organisms. Pfitzer (63) has raised the objection to it, that the velocity of osmotic currents would be so slow as not to cause motion. This is certainly a valid objection, notwithstanding the explanation of Kozlowski (43) that "the cause of movement does not lie in the velocity of the current, but exclusively in the change in position at the centre of inertia, and is equally true whether liquid flows out through macroscopical or molecular openings." Hansgirg's efforts (35) to show such currents were made by placing *Oscillaria* filaments in almond oil. In this way he found that there was a delicate pellicle of water surrounding the organism which was sufficient in some cases to cause motion for four days, when the layer of water would finally be broken up and the movements cease. Such an experiment, it would seem, would be a stronger argument against osmotic currents than for them, notwithstanding the efforts of the supporters of this theory to make out that the movements are very slight, even if they *look* very great on account of the magnification used. When we magnify the movements we also magnify the organism, and the relation between the two remains the same. Thus how the very thin pellicle of water which remains surrounding the trichomes when placed in the almond oil, can be sufficient to set up osmotic currents of such magnitude as to cause motion, either oscillating or creeping, of several times the diameter of the plant, is difficult to understand, as is likewise how the delicate, hydroscopic pellicle on the trichomes of the Oscillarieæ growing on moist soil can cause movements over the surface of the supporting substratum as claimed by this author, for the delicate film of water surrounding them as they creep is many times thinner than the diameter of the trichome, though the motion passes through many times that distance.

The ingenious ideas of Wolle and some others concerning these movements being caused by the multiplication of cells, are also similar theorizings without any basis in observation. In one place Wolle claims that the cells begin dividing on one side and this concert of division would cause that side to become longer and cause a flexion of the trichome or the oscillatory motion. In another place he considers the creeping motion to be due to the growth and multiplication of the cells in the trichome. But as this creeping motion is in no way accompanied by the oscillation or undulatory movement which the one-sided splitting was supposed to cause, the cells would have to divide evenly on both sides, instead of from alternating sides as the first part of his theory supposed.

Here is where observation shows us the fallacies that theory alone may build up. The facts are, these cells do not begin to divide on one side first, but equally on both sides. Moreover, aside from the mathematical alternation from side to side, which this theory supposes in the divisions, and the unheard-of rapidity with which they must occur in order to account for the backward and forward oscillations, if there should be an organism in which the partition walls were laid down from one side, it would differ from any other known cell, and be contrary to the laws of physics if it were to swell out that side of the cell more than the other. Further, observation also shows us that the trichomes move fastest in the daytime, while the cells multiply most rapidly in the middle of the night. Even in the night when the growth is most rapid and the motion almost stopped—probably would be completely so were it not for the light necessary in process of observation—the growth can scarcely equal the rate claimed by Wolle and his followers. Another point overlooked by these investigators, is that both ends of the trichome move in *the same direction and at the same rate of speed,* which would be impossible if the multiplication of cells caused the movement.

Strasburger (75) said: "Simultaneously the threads show irregular flexions, or nutations, which are the expressions of the existing differences in the intensity of growth on the different sides." In order to account for the rapidity of this motion being greater than could possibly be caused by the one-sided splitting of the cells of the trichome, Strasburger continued, "These flexions usually take place slowly, but can, however, induce violent movements when the flexion is stopped by some obstacle, and then by overcoming this the tension is suddenly equalized. The movements can only take place when the threads have a point of support on some other object." This last statement is contrary to the observations of Engelmann, as well as my own, as will be shown later. The evidence of nearly all observers on the hormogonia would also militate against it.

The observations of Schultz and his followers seem much more to the point. Such movements occur nowhere else among living organisms without the presence of some kind of locomotory apparatus, usually cilia in some form. In the light of the facts above stated, it would seem, therefore, that we have only two methods left as plausible explanations of the motions of the Cyanophyceæ, *i. e.,* either (*a*) a protoplasmic pellicle which creeps along on a substratum and which acts in a peristaltic manner, or (*b*) the plant has some propelling organ as flagella or cilia or pseudopodia, that act either upon the solid substratum or that move freely through the water. The former of these theories could scarcely account for all of the activities, though it might assist in them. While studying the species of *Oscillaria,* I was convinced that there was, at times, a very delicate pellicle surrounding the trichomes. This pellicle, as is shown above, would take a delicate tint with protoplasmic stains. On several occasions I saw a lengthening and shortening of the cells as in peristaltic movement, but it was by no means sufficient to cause the oscillation and especially the creeping movements of the organism. Neither was there any observed evidence that the peristaltic movements, if such they could be called, were caused by the surrounding delicately staining sheath, even though it should prove to be protoplasmic, instead of the gelatinized outer lamellæ of the sheath, as is held above.

The presence of long, hair-like appendages from the terminal cells of *Oscillaria* have been mentioned by several observers. Borzi (5), Hansgirg and Gomont have described them, but have interpreted them as parasitic organisms. Hansgirg (35) spoke of them as follows: "The so-called cilia with which the end cells of many species of *Oscillaria* are provided, are independent leptothrix-like organisms, belonging to the family *Ophiothrix*." Concerning the development of these organisms, Hansgirg continued: "While I observed the creeping motion of the filaments of *Oscillaria princeps*, and examined their anatomical structure more closely, I was not surprised one day when I noticed in the open ends of one of the dead filaments, many small amœboid cells, mostly only nine to twelve microns in diameter in the outflowing protoplasm. From these cells I noticed, after they had separated themselves from the general mass, that colorless pseudopodia, arranged in ray-like fashion, were produced and increased in size."

The appearance of these long hair-like trichomes on the terminal cells of different species of *Oscillaria*, and on the terminal cells only, cannot but arrest the attention of the most casual observer. They can easily be seen with as low a power as Zeiss' AA objective, and for some time I was of the opinion that they might be parasitic organisms as asserted by Hansgirg and Gomont (31), but upon following them closely, I was convinced that this was a mistake and that they are a definite part of the plant, and have some definite function in the plant economy. If they appeared on any other portion of the trichome besides the terminal cell, it might be easier to consider them as parasites, for their function is not very evident, but there seems no reason why they should select this one cell as the point of attack, for it is no more vulnerable than any other cell, and in fact is often protected by means of a calyptra. Hansgirg's contention that they arise from amœboid bodies in a broken cell is not to the point, because they often arise in unbroken end cells. Further, Hansgirg considered that the long hair-like appendages along the sides of *Cylindrospermum* were of the same nature as the hair-like organs of *Oscillaria.* But since these long processes only appear upon the sides of the heterocyst and spores, both of which have strongly thickened walls, it can scarcely be thought that any parasitic organism would penetrate these, and not be found upon the thinner-walled vegetative cells. I therefore placed some of these filaments in a live-box and kept them under continuous observation until these hair-like organs were completely formed, which usually consumed from one to three hours, according to the strength of the culture and other conditions involved. Observation was continued for several days, and I was rewarded many times by being able to trace the gradual development of these so-called parasitic growths from the protoplasm of the end cells, noting them in all stages of their growth from small swellings to the full-grown hairs. When a trichome is broken across, the final cell of each broken end exhibits the normal structure (Fig. 39). Gradually the chromatin of the central body becomes diffused and the free end bulges out, on account of the turgor and relieved pressure from the other cells, on one side (Fig. 33). As this proceeds, small finger-like processes begin to appear as small swellings over the free surface of the cell. These gradually grow out until they become eighteen or twenty times as long as broad. In their younger condition, they take the stains in the same manner as the protoplasm of the end cell, but gradually they become more impervious to these stains and react in the same manner as the cell walls, except for the thin protoplasmic continuation into their lumen. Thus it will be seen that they slowly harden into a cell wall by the deposition of some substance which no longer takes the stain as the softer protoplasm does. They gradually assume the reaction of cellulose.

which they retain but a short time, finally reacting to all tests in the same manner as the cell wall of the older portions of the Cyanophyceæ, with which they become continuous.

What the function of these hairs is, I have not been able fully to determine. That they are not parasitic growths, however, but living portions of the *Oscillaria* filaments which have grown out from the cell protoplasm, either through pores specially digested out of the free cell wall by means of a ferment, or more likely through the pores formerly occupied by the protoplasmic communications between the cells (Fig. 35), there can be no doubt. That they act more or less as a tactile organ, assisting the trichome to overcome and get around obstacles met with in the forward progress of the filament, was evidenced many times in the progress of my observations, for I was able to see them swaying from side to side, with a slow but steady independent motion as the *Oscillaria* filaments moved forward or oscillated. They would apparently reach out on one side as if to attach their free ends to something, and then gradually sway over until they became bent in the opposite direction. If the trichome pressed forward against some other object or *Oscillaria* trichome, these hair-like appendages would, through the activities just mentioned, apparently determine the way to get around the obstacle, as if they were tactile organs that piloted the trichome and assisted it in surmounting obstacles. These actions were so general and constant that, though I do not consider them to be rapid enough to cause the oscillation or creeping movements, nevertheless, they were such as to convince me that I was dealing with a definite plant organ. Having determined this, I sought after an adequate cause for the movements observed. As has been said above, nowhere in the whole vegetable kingdom, do such movements, the cause of which is understood, occur without ciliary or pseudopodial appendages. Various stains and micro-chemical tests were tried, but without marked success. Aside from a decided roughness of the outer contours, nothing could be seen for a long time. The long hair-like trichomes which pass out from the walls of the heterocysts and spores of *Cylindrospermum* had been seen, but they did not have the proper movements to cause the entire motion under investigation. By Gardiner's corrosion method with iodine and sulphuric acid, I was able to plasmolize the protoplast of the spore of *Cylindrospermum* and the trichome of *Oscillaria* very greatly (Figs. 27 and 63), so that the shrunken protoplast showed very plainly a number of finger-like processes that passed out through the spore walls and became continuous with these hairs. The corrosion had so swollen the cell wall, that the minute pores through it were very evident. These hairs, then, are not parasitic organisms as supposed by Hansgirg, though they did not seem to cause the motion of the plant except, probably, to determine the direction in which the plant would move, or to cause the forward end to avoid an encountered obstacle.

Remembering that the spores and heterocysts of these plants are but modified vegetative cells, it is easy to perceive that these pores through the spore wall must have been present in the vegetative cell, though difficult to demonstrate. This I was able to show, and the long hair-like appendages, which Hansgirg had supposed to be parasitic organisms, are in reality, stronger growths of the fine cilia which cover the sides of the trichomes. The spores finally lose their hair-like appendages and the pores become closed through the thickening of the wall. Realizing that the cilia along the sides must be very delicate, I employed methods of staining which are used to demonstrate flagella on the bacteria. By the use of Bunge's mordant, followed by carbol fuchsin, which brings out the flagella of the bacteria very nicely, I was able to demonstrate that the roughness of contour which I had noticed with other stains, was in reality caused by tiny protoplasmic knobs (Fig. 40) which were continuous through the cell wall with the protoplast within. Radiating lines of a deeper staining protoplasm or kinoplasm always connected the points of egress of these protoplasmic knobs with the central body. Careful study of these peripheral balls convinced me that they were not in their normal form. I therefore varied my method of mordanting and staining and finally found that these minute balls were very delicate cilia that had been massed down against the outer wall of the plant. They stained with all of the ordinary protoplasmic stains, but were so delicate that the least amount of washing for differentiation deprived them of their color. These cilia are so small that it is with extreme difficulty that they can be seen in the living organism, and the probable reason that other observers have overlooked them is that they, unlike the flagella of the bacteria, mass down when placed in reagents and appear as granules of foreign substance on the exterior of the trichome. They have quite the appearance of tactile organs, and in fact for a long time I mistook them to be such, before I was able to demonstrate that they were the massed substance of the cilia. These facts will explain more clearly the moving of the particles of indigo along the trichome as described by Schultz and others, and the massing down of the cilia will explain why the contour of the trichomes is so rough oftentimes, as is especially shown when stained with Heidenhein's iron-ammonia-alum hæmatoxylin, with but slight or no destaining. Engelmann was able to show this same granular roughening of the contour of the filament of *Oscillaria,* but did not see the cilia which caused it. By the use of Gardiner's method of corrosion, I caused the protoplast of the isolated cells of *Oscillaria* to so shrink as to withdraw these delicate projections of the protoplast and expose the minute pores in the swollen wall (Fig. 27). Figure 26 shows a trichome thus treated, in which the protoplasts have shrunken away from the cell walls, but evident points or processes pass out towards each of the pores through which the cilia passed.

Fischer (28) found membranous projections passing from around the central body into the surrounding chromatophore. He considered them to be continuous with the protoplasmic membrane around the chromatophore (Figs. 29 and 32 are copies of his drawings). These membranous projections, I think, are evidently the prolongations of the ground mass from the central body or nucleus, to the cilia, though he failed to see them pass the cell wall (compare Figs. 29 and 32 with Figs. 31 and 38). I have, with Engelmann, been able to see *Oscillaria* and *Cylindrospermum* swimming freely in the water, which is beyond the power of pseudopodia to cause. It is therefore evident that the creeping movements of these organisms are effected by the action of definite, very delicate, short cilia. These were demonstrated around the wall of the cells in a more or less uniform number (compare the various figures).

The contention of former writers that motion in the Cyanophyceæ was caused by light, heat, etc., does not in any way militate against the views here taken. The effects of these stimuli are but the workings of the ordinary laws of biology. Wigand (84) showed that light was a prime factor in the protoplasmic movements of the higher plants, and in the Cyanophyceæ we find the same thing to be true. Movement is much stronger in the light than in darkness, and it is probable that if they could be observed in total darkness, there would be no motion at all. The action of heat and cold was also the same in these organisms as in the higher cells. By means of Reichert's warm stage, I observed these organisms under varying conditions of temperature and found that they had their maximum, optimum and minimum temperatures, just as found in other plant cells.

(8) CONCLUSIONS.

The line of demarcation between the Cyanophyceæ and the other algæ has been based upon the threefold negative characteristics: (*a*) absence of sexual reproduction; (*b*) absence of a chromatophore, and (*c*) absence of a nucleus. Inasmuch as many higher plants having undoubted cell nuclei are devoid of sexual reproduction, it seems that this is scarcely a proper characteristic upon which to base a distinction. But even this is represented in the Cyanophyceæ by the flowing of what may be hereditary material through the intercellular protoplasmic threads in the formation of spores. Sexuality, after all, is merely the passing of certain reproductive or hereditary substances from one cell into, and its fusion with, those of another. This is certainly accomplished by these plants, and probably only during the formation of the spores or reproductive bodies. On the other hand, the chromatophore and nucleus are undoubtedly present. The nucleus has begun to differentiate the karyokinetic division as is found in higher plants, but has not reached the same degree of evolution. The chromatophore is also primitive, and combines the function of a color-bearing organ with that of the cytoplasm. Thus the boundary which has seemed to separate the Cyanophyceæ from other algæ has been entirely removed, and they are shown to be merely a more lowly organized form of true algæ, having many characteristics in common with them.

There can be no doubt, however, that these plants are much higher in their organization than has been supposed. Their evident correlation of movement, their intercellular protoplasmic continuity, their sexually formed spores, and many other activities, place them much higher in the scale of classification than we have supposed, though they still, in various points, exhibit the beginnings of the activities which we find in the higher plants. These facts have been shown by the present investigation and have been reported fully above. They are briefly reviewed in the following summary:

(9) SUMMARY OF RESULTS.

1. The central body of the Cyanophyceæ is composed of chromatin and is a true cell nucleus.

2. This nucleus divides by one of two methods, both of which start upon the karyokinetic history, one going no further than the net-spireme stage where it constricts itself into halves, while the other continues further and forms a rudimentary spindle with rudimentary chromosomes upon linin threads.

3. In both forms of division, the nucleus divides itself, not being strangled into two parts by the ingrowing partition wall.

4. The chromatin is arranged on the spireme thread in granules which multiply in number by transverse divisions.

5. There is no longitudinal splitting of the chromosomes or of the spireme, and in the division of the cell by the method first mentioned above, the two portions of the nucleus are not necessarily equal.

6. The chromatin is aggregated in hollow vesicles in the resting cell. These vesicles give out their chromatin to the net-spireme very much like the nucleoli of higher plants, and they may represent it. They are imbedded in a granular ground substance.

7. The outer zone of the protoplast is divided into two portions, a thin colorless ectoplasm lining the cell wall, and the thicker layer between it and the central body. This latter portion contains the pigments which are dissolved in it, and is rightfully termed the chromatophore.

8. The cyanophycin granules and slime balls are probably food products. They are located in the chromatophore.

9. The movements of *Oscillaria, Cylindrospermum* and the other forms of the Cyanophyceæ which exhibit motion, are caused by delicate protoplasmic cilia distributed along the sides of the trichome.

10. Finger-like processes of the ground mass of the

nucleus radiate out toward the periphery of the cell, piercing the chromatophore and cell wall, and project in the form of the cilia which cause the movements of the trichomes.

11. The finger-like processes upon the end cells of *Oscillaria,* and those surrounding the heterocysts and spores of *Cylindrospermum,* are not parasitic, but definite organs of the cell, having a motion of their own. They apparently assist the trichomes to pass around obstacles.

12. The protoplasts of the cells of filamentous Cyanophyceæ are all connected by fine protoplasmic threads which pass through communicating pores in the walls. There is usually one central pore, though other finer pores and threads may be present.

13. The heterocyst is a modified vegetative cell which gradually fills with some substance, passed to it from the other cells, through the pores for the protoplasmic threads which connect it with the other cells of the trichome. This substance finally fills the whole of the heterocyst. It gives some of the reactions for chromatin and may be a modification of that substance. The heterocyst of *Cylindrospermum* will develop into a spore if it gets sufficient nutriment and hereditary material passed into it from the other cells.

14. Spores are formed in *Oscillaria* from groups of cells, usually two, but it may be one, three or four. These fuse by the absorption of their partition walls. The growth of the spore is effected by substances passed into it from the other cells. The spores of *Cylindrospermum* are formed from a single cell which divides, the end cell becoming a heterocyst and the second cell the spore. It also receives substances from the other vegetative cells.

15. The cell wall is composed of cellulose in its earlier stages, but later becomes impregnated with or modified into some substance akin to fungus cellulose.

16. The cell wall is laid down as microsomata, in lamellæ on the inside of the cell wall. One such lamina is laid down at each division. Thus every succeeding lamina from within

outward will surround twice the number of cells as the preceding one, as shown in Fig. 94.

17. In *Oscillaria* the outer laminæ are dissolved off by the water. In *Nostoc* they swell collectively into a jelly which is permanent, while in *Lyngbya* the jelly remains as it was deposited, in thin tough layers.

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(11) EXPLANATION OF PLATES.

Plate XXIII.

Fig. 1. Cell of *Oscillaria* showing the chromatin vesicles of the central body in the resting condition. Also shows the cilia on each side wall.

Fig. 2. Diffuse net-spireme stage of division in *Oscillaria.*

Fig. 3. Net-spireme drawn to the equator of the cell of *Oscillaria.*

Fig. 4. Net-spireme constricting and division walls of consolidating microsomata growing inward from the side walls.

Fig. 5. Division of cell of *Oscillaria* completed.

Fig. 6. Cell of *Oscillaria* with diffused nucleus, from which the net-spireme of Fig. 2 will form.

Fig. 7. Cell of *Oscillaria* showing the beginning of the collar-like ingrowth, which will form the division wall. Ingrowth composed of microsomata of cellulose.

Fig. 8. Spireme forming into segments or chromosomes in *Oscillaria.*

Fig. 9. Same as Fig. 8, but with chromosomes and spindle fully formed.

Fig. 10. Same as Fig. 9, but with the chromosomes dividing.

Fig. 11. Cell of *Oscillaria* in which two large chromosomes have formed.

Fig. 12. Same as Fig. 10, but with heavier chromosomes.

Fig. 13. Chromosomes shown in Fig. 11 are here shown dividing.

Fig. 14. Division completed.

Figs. 15-20. Successive division stages in *O. Froehlichii.*

Fig. 21. Spireme of *Oscillaria* much enlarged.

Fig. 22. Portion of Fig. 21, more highly magnified, showing the chromatin balls.

Fig. 23. Same as Fig. 22, but showing the chromatin balls dividing.

Fig. 24. Chromatin vesicles crushed out of a cell of *Oscillaria.*

Fig. 25. Portion of filament of *O. Froehlichii* treated with iodine and sulphuric acid. The protoplasmic threads are shown, which connected the end cells with the neighboring protoplasts.

Fig. 26. Portion of filament of *Oscillaria* plasmolized with 95 per cent. alcohol. Shows each point of the contracted protoplast directed toward an opening in the cell wall through which it passed.

Fig. 27. An isolated cell of *Oscillaria* treated with iodine and sulphuric acid and viewed from the "end." Shows the openings in the cell wall and the finger-like processes of the protoplast which passed through them.

Fig. 28. Isolated cell of *Oscillaria* viewed from the end. The central body here has assumed a deeply staining granular, irregular condition, which probably precedes the formation of the heavy chromosomes.

Fig. 29. Copy of Fig. 42 in Fischer's "Untersuchungen ueber den Bau der Cyanophyceen u. Bakterien." This figure shows what Fischer considered to be radiating membranes from the ground mass of the central body. Compare with Figs. 38 and 40.

Fig. 30. Cross-section of *Oscillaria* trichome, which has been cultivated in a full nutrient solution. The chromatin vesicles are very large and chromatic. The radiating lines from the ground mass of the central body are also accentuated.

Fig. 31. Cross-section of *Oscillaria* trichome, which shows the chromatin vesicles of the central body, and the kinoplasmic threads radiating from it to the cilia.

Fig. 32. Copy of Fig. 44, from Fischer's "Untersuchungen ueber den Bau der Cyanophyceen u. Bakterien." This figure shows in cross-section what Fig. 29 has in side view. These radiating membranes are evidently the radiating kinoplasmic threads which pass from the central body to the cilia.

Figs. 33-36. Successive stages in the formation of the long hair-like organs on the end cells of *Oscillaria.*

Fig. 37. Portion of trichome of *Oscillaria* grown in full nutrient solution. The chromatin vesicles are greatly enlarged.

Fig. 38. Portion of filament of *Oscillaria* showing the radiating lines from the central body or nucleus, and the cilia along the sides.

Fig. 39. Same as Fig. 38. Note the characteristic irregular arrangement of numbers of cilia on each cell.

Fig. 40. Same as Figs. 38 and 39, but showing the cilia massed down by the action of the reagents.

Fig. 41. Portion of filament of *Oscillaria* showing cross lines on the cell walls.

Fig. 42. Portion of filament of *Oscillaria* showing longitudinal lines on the cell walls.

Figs. 43-44. Portions of filaments of *Oscillaria* in which the end cells have been pressed away from the second cells, exposing minute protoplasmic threads connecting the protoplasts.

Fig. 45. Growth of a spore of *Oscillaria.* The old spore wall or exosporium still remains.

Figs. 46-50. Development of a peculiar amœboid-like spore and its formation of a new *Oscillaria* trichome. See page 304.

Figs. 51-52. Formation of spores by the fusion of the cells. The darker cells act as "nurse cells" to feed the spore-forming cells. The successive stages are lettered from a to h.

Plate XXIV.

Fig. 53. Full-grown trichome of *Cylindrospermum,* showing cilia and hair-like appendages.

Fig. 54. Cross-section of spore of *Cylindrospermum.*

Fig. 55. Full-grown spore of *Cylindrospermum* before withdrawal of appendages.

Fig. 56. Double heterocyst in *Cylindrospermum.* Shows the gelatinous envelope frayed out into finger-like processes at the end.

Figs. 57-62. Successive stages in the formation of the spores of *Cylindrospermum.*

Fig. 63. Spore of *Cylindrospermum* treated with iodine and sulphuric acid, showing the plasmolized protoplast with the finger-like processes which passed out through the spore walls.

Fig. 64. Trichome of *Cylindrospermum* in which a second spore has formed.

Fig. 65. Copy of Zukal's figure, which shows hair-like flagella from the partition walls of *Cylindrospermum.* The different sized bodies crushed out of the cell are what he terms gametes.

Figs. 66-73. Successive stages in the karyokinetic division of *Cylindrospermum.*

Fig. 74. Highly magnified end of trichome of *Cylindrospermum,* showing the spore and heterocyst with their long finger-like processes and the gelatinous sheath frayed out into shreds. The pores through which the protoplasm passes to connect the protoplasts are also shown.

PLATE XXV.

Fig. 75. First stage in the formation of the heterocyst of *Nostoc.*

Figs. 76-79. Successive stages in the formation of the heterocyst of *Nostoc.*

Figs. 80-83. Four successive steps in the formation of the net-spireme and division of *Wollea saccata.*

Figs. 84-86. Different steps in the formation of the spores of *Wollea saccata.*

Figs. 87-89. Stages in the division of *Anabæna flos aquæ.* The steps follow the order of the letters from a to h inclusive.

Figs. 90-92. Colonies of *Glœocapsa polydermata,* showing the cell wall surrounding different generations of cells. Evidently here there are more laminæ laid down after each division than is found in other forms.

Fig. 93. Cells of *Nostoc* slightly disintegrated, showing the long protoplasmic connections between the cells.

Fig. 94. Trichome of *Spermosira litorea,* showing how the sheath is formed by the deposition of layers of substance on the inner face of the parent walls. As division proceeds, these walls are made to hold different numbers of cells, as described in the text.

The Structure and Relation of the Plastid.

(*WITH PLATES XXVI, XXVII.*)

BY CASSIUS H. WATSON, B. S.

In the study of the morphology and physiology of the plant cell, great advances have been made during the past few years in the investigation of the structure, division and distribution of plastids in different plants. The old definition, "plastids are differentiated portions of the protoplasm, which, like the nucleus, are not formed *de novo,* but multiply by division," is so far satisfactory, and may serve as a starting point from which to investigate other questions bearing on the plastid. The above definition gives no suggestion as to the genesis of the plastid, and it is to the study of this question that the present paper is devoted. The study was undertaken at the suggestion of Professor Macfarlane, who alike in his paper on *Dionœa* * and in his graduate lectures has shown that some plastids are connected with the cell nucleus by chromatin threads, and exhibit a minute structure that seems to be identical with the nucleus of the cell to which they are organically attached.

From the investigations of Schimper, Meyer and others we now know that plastids exist in the cells of the embryo plant even before the seed-coat has ruptured. All evidence is in favor of the view that these are descended from the cells of the parent tissue, out of which the sex-cells were organized; a similar relation seems to hold true for the sex-cells and spore-cells of the lower plants.

But in order to ascertain regarding the genesis of the plastid, it seemed possible that a comparative study of them or their supposed homologues in a series of plants, beginning with those low in the scale of evolution, and ascending to higher and more specialized types might prove suggestive.

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*Botanical Contributions, University of Pennsylvania, Vol. 1, p. 35.

Collection and Fixation Methods.—As a simple and useful material, I was fortunate in discovering on some *Nitella* an abundant growth of a parasitic *Coleochaete* (*C. soluta*). Alcohol of 30 per cent. strength was used as a killing fluid, and the percentage was gradually increased up to 70 per cent. Similar treatment was adopted for *Oscillaria, Cladophora* and *Zygnema*. Different fluids were used for *Spirogyra*. After repeated experiments, mercuric chloride proved most satisfactory. In the use of this agent, subsequent staining was unnecessary, as sufficient differentiation was secured by the action of the mercuric salt. For *Nitella,* weak alcohol proved most satisfactory. Gardiner's sulphuric-acid-iodine method for the demonstration of protoplasmic continuity proved of value, but a weak solution only of the acid was used for a short time.

Anthoceros was killed in several fluids, the most satisfactory being Flemming's compound and 1 per cent. chromic acid. Of the two chromic acid is preferable, as giving the clearest and least altered details. Among the mosses *Funaria* was chosen, and the best results were secured with 1 per cent. chromic acid. A variety of fluids was used on fern prothallia, and sulphuric-acid-iodine treatment gave very pleasing results. The delicate prothallia, after being killed in Flemming's fluid and subsequently washed, were dipped quickly in dilute sulphuric acid solution, then washed and placed in iodine solution, rewashed, stained and mounted in 1 per cent. acetic acid.

Of the Lycopodineae, *Psilotum* was selected on account of its large nuclei and plastids. Various methods were used, Flemming's fluid serving best, as a fixative. Serial microtome sections were also prepared in the usual manner. Amongst Monocotyledons, *Cypripedium insigne* and *Dieffenbachia* were chosen, owing to the large size of the plastids.

Observations.

Zygnema.—Each cell of *Zygnema* possesses two pyrenoids suspended within the lumen of the cell by means of strands of a clear substance. Each pyrenoid consists of a very dense, deeply staining nodule; while around it is a less densely staining area of a granular character.

In Fig. V this is clearly shown, and in some cases the granular character extends a distance into the suspensory strands. Midway between the two pyrenoids is seen a clear refractive body, the nucleus, not staining as deeply as the pyrenoids, and embedded, as it were, in the substance of a broad band of cytoplasm joining the two pyrenoids. This body is constant in all cells in the normal resting stage and always in this position. It is evidently associated closely with the active areas on either side of it.

Fig. III shows the condition after conjugation is completed. The four pyrenoids are distinctly shown suspended by filaments from the wall of the aggregation capsule.

Fig. IV shows division of a pyrenoid. The two dense bodies formed by fission of the parent pyrenoid gradually separate, while between them is a clear band in the center of which will later appear the nucleus which for the time is not observable. In comparative structure, relative stainability and close association the pyrenoids and nucleus of *Zygnema* suggest a close relationship.

Spirogyra.—The most available type chosen was *S. nitida* and the minute structure of the chlorophyll bands was first studied. Running in the center of the band, as shown in Fig. XV, is a continuous clear strand about 5 microns in width at its narrow portions. At intervals in this apparently clear spiral strand appear dilatations into sac-like structures. The sac-like portions are budded off from each other by constrictions, then by growth of the intermediate portion, due to the constriction, the two sacs become separated.

Within the lumen of each sac rests the so-called pyrenoid body, not free within the cavity, but suspended at or near the center by means of threadlike prolongations of the bulb capsule. The structure of this pyrenoid is more dense than that of the axial strand and seemed almost identical with the same bodies in *Zygnema.* This strand and its specializations lie embedded in the mass of green chlorophyll constituting the greater part of the spiral band. Between adjoining chlorophyll bands delicate, slightly granular strands cross the interspace between two adjacent bands. It was impossible to determine whether these came from the axial portion or were prolongations of the chlorophylloid band substance. A well-known feature of *Spirogyra* is the great number of strands suspending the nucleus in the cell cavity. On careful observation it is seen that these suspensory strands are of the same material and in every way identical with the substance of the axial filament.

Fig. XV shows an ending of one of these filaments, and while the exact continuity of the axial with the suspensory strands is not apparent, from the nature of the substances present, that is the only conclusion that seems likely.

Cladophora.—In many respects this plant resembles *Spirogyra.* Here, however, the axial filament is entirely absent, and consequently we have a more diffuse, irregular distribution of the chlorophyll masses. Branching processes of the material in a rather granular condition ramify over the peripheral protoplasm. Scattered about in these processes are the pyrenoid areas, with no attempt at systematic distribution (Fig. II). Along with these are smaller deeply staining bodies that resemble the pyrenoids.

The same structures are present as in *Spirogyra,* except the axial filament; there being a densely staining body suspended by very delicate strands from the walls of an enclosing hyaline capsule. Many authorities describe the smaller of these bodies as the nuclei (each being polynucleate). and yet they appear to be identical in structure with the pyrenoids of *Spirogyra.*

Vaucheria, Oscillaria.—In the former the chlorophyll is distributed in plastids of varying size in the cell protoplasm. In *Oscillaria* the condition is still more primitive, and the chlorophyll green is diffused throughout the protoplasm with no attempt at localization.

Coleochaete has a similar diffuse chlorophyll structure. The nuclei stain densely, of a density similar to that of pyrenoids, and in many instances show small refractive nucleoli. These nuclei are situated at or very near the center of the cell and are surrounded by a rather clear area. This area, however, is not bounded by a membrane of any kind.

The nuclei of *Coleochaete* suggest in their appearance the pyrenoids of preceding plants, while their contained nucleoli resemble, in their strong refraction and density, the nucleus of *Zygnema.*

Nitella.—In this plant the plastids are arranged in long parallel rows close beneath the cell wall and are held together against the force of circulating protoplasm by branching strands, which place the whole system in communication. There seems to be no regularity of branching of these strands, the whole merely forming an irregular network. The strands take the eosin stain slightly, but owe their distinctness to their refractive character, and also to the granules adherent to their substance.

The plastids are of a uniform, quite densely staining character and divide by simple fission. Fig. X (d) shows a plastid dividing; while (s) represents two plastids just after completion of the septum.

Anthoceras.—The plastids of this plant are joined together by interplastid fibres and form a general network system as in *Nitella.* The plastids are of a large, quite densely staining character, and exhibit a finely granular structure. Along the interplastid fibres are distributed numbers of fine granules seemingly of the same nature as those within the plastid (Fig. XII).

The nuclei are of large size and their substance of a coarsely alveolar nature, within is a very densely staining nucleolus. A number of the plastids are observed to contain a single body, each of the same general size, density and staining quality as the nucleolus. Indeed, without other evidence, this is the only fact that would cause suspicion of relationship between the nucleus and the plastids.

The plastids divide by fission as is shown in Fig. XII (d), where the constriction is just beginning.

Funaria hygrometrica.—The thin new leaflets stained in eosin and mounted in balsam were used. The large rather flattened cells near the margin of the young leaves toward the tip were most favorable for study.

The plastids are large and stain deeply, they are made up of a granular or alveolar structure quite similar to the nucleus.

The multiplication is by simple fission, and in cells in this position it will be seen by Fig. VII that divisions go on very rapidly.

It will also be seen that the plastids are joined together by fibres, and that in some cases, for example, Fig. VII (s), a definite system has been formed, *i. e.,* we can easily understand how a group of plastids might arise from one parent through successive divisions, and that these might all remain joined by filaments which persist as the attenuated connecting strand formed when two plastids drew apart.

Prothallus of Fern (Adiantum).—The plastids are packed quite closely. The nuclei are but little larger than the plastids. In structure, both plastid and nucleus are identical, though the nucleus probably stains slightly the deeper.

The connecting fibres show no definite system, owing to the great number of divisions that take place. They divide by amitosis as in Fig. XVIII (a), (b), (c).

Fig. XIII shows a peculiar type of plastid occurring in the subepidermal tissue of *Pteris bicolor.* A limited number of these occur in the different cells. They closely simulate nuclear characters, bands of substance winding in a meridional or parallel manner about the plastid and staining very like chromatin. Possibly this indicates a trace of nuclear character occurring in certain specialized plastids of the ferns.

Fig. VI represents plastids and nucleus from a deeper level, also in *Pteris bicolor.* The plastids here stain densely, and show decided tendency to grouping by connecting threads. The nuclei are of finely granular type and bear from one to several nucleoli.

Psilotum.—This plant proved to be one of the most advantageous of the materials used. Here, no doubt, a large part of the success was due to varied methods of killing, mounting, etc. I found, as stated under "Methods," that Flemming's fluid was the most satisfactory killing agent, the specimens being subsequently stained and mounted in both acetic acid and in balsam. Of these the acetic acid proved clearest as to detail.

The plastids are of two sizes, the large deeply staining ones occurring in the subepidermal tissue, and the smaller less dense type, that is scattered through the deeper cells, making up the greater portion of the substance of the plant. Both nuclei and plastids present the same rather coarsely alveolar structure.

The subepidermal cells for about four, five and six layers are quite crowded with plastids. In these cells the structures are so obscured that I have made my drawings from the deeper, more elongate cells with fewer plastids. The nuclei in both types of cell are of large size, of an alveolar nature and show a distinct bounding membrane.

Figs. IX and XIV show the plastids grouped in the deeper tissues. Here is present the arrangement that is so evident in the mosses, indeed it seems more striking in this type than in any other examined.

Fig. VIII is an enlarged drawing of the nucleus of the cell shown in Fig. XIV (a). The group of plastids is a diffuse type, *i. e.,* the new plastids formed are budded off in no regular direction or sequence. The interplastid fibres are shown joining them.

The group of three plastids marked P_1 would seem, at first sight, to have come from the larger plastid (s), to which they are all attached. Such, however, is probably not the case. On closer examination, each of the three is in its turn joined by fibres. Thus either they came from the large one (s), or were budded off from their adjacent predecessors. In either case there is left necessary an explanation of the interplastid fibre. This question will be treated in the conclusion, so it suffices here to mention it.

There are also conspicuous fibres joining the plastid system to the nucleus. Within the plastids are clear refractive bodies. Fig. VIII (P_3 and P_4) shows two stages of division of this body, and, judging from these and others, I believe this dense refractive body is a definite element in the make-up of the plastid.

Cypripedium insigne.—The plastids here are of a rather irregular outline and stain densely with eosin. The drawing, Fig. XI, represents them as structureless; but they have a finely granular appearance, that contrasts with the represented granular protoplasm.

No interplastid fibres are shown, but since making the drawing I was enabled in an acetic acid mount to make out very distinct strands of a marked refractive nature joining the plastids together.

The systems are quite plain, but seem much more diffuse and less easily isolated than in such plants as *Funaria.* The observations were made on freshly cut leaf sections mounted in water, also killed and mounted in acetic acid.

Dieffenbachia.—The plant was chosen to represent a high type of specialization, and proved valuable material. The subepidermal tissue in this is very abundantly supplied with plastids, while in the deeper tissue, that stores masses of starch, the plastids are much fewer and of a smaller, less dense character. The interplastid fibres are very numerous and form a complex network within the cell. Fig. XIV (sx_1 and sx_2) also (sl_1 and sl_2) seems to show what might recall an attenuated condition as seen so completely in *Psilotum,* but these fibres undoubtedly extend in many directions and constitute a far more complex condition than I have represented.

CONCLUSIONS.

From a study of the above types it appears that while in the simplest organisms the chlorophyll is diffused through wide areas of the protoplasm, it becomes more and more restricted in the higher type to special bodies, the plastids, that exhibit in most cases a structure closely resembling, if not identical with, the nucleus of the cell in which these plastids lie. Further, definite refractive threads, that greatly resemble attenuate chromatin, link together the plastids with each other, and with the nuclear membrane. The resemblance between the pyrenoids and nucleus of *Zygnema* and *Spirogyra* as to general morphology, stainability and relationship is suggestive. In the higher plants, from *Funaria* upwards, the connection between plastids and nucleus is evident, while striking resemblances in their finer histological details are undoubted. It seems therefore not unnatural to suppose that plastids primarily represent nuclear differentiations of the cell, which have been separated off for the special purpose of metabolizing special fruit constituents, the nucleus in the process being left as the special directive centre of each cell. While it may be extremely difficult to secure positive proof of this proposition, many observational results strongly point in the direction indicated, and the accumulation of additional evidence for and against the present views is much to be desired.

The Relation of Ice Storms to Trees.

By John W. Harshberger, Ph. D.

The year 1902 was noted for two exceptionally destructive ice storms that visited the region lying contiguous to Philadelphia. One of these storms occurred on Friday, February 21st, and the other on Saturday, December 13th. The storm of February 21st was accompanied by high winds and did an irreparable damage to the fruit, forest and shade trees. The storm of December was noted for the larger amount of ice formed, but the damage was not so great, because there was very little wind to break off the limbs that were weighted down with the ice.

Meteorologically speaking, regions of strongly variable temperature are subject to occasional winter storms in which the precipitation, occurring as rain, freezes as soon as it touches any solid body, such as the branches of trees, telegraph wires or the ground. This happens when the ground and the lower air have been made excessively cold during a spell of clear anticyclonic weather, when a moist upper current in advance of an approaching cyclone[1] brings clouds and rain. New England is particularly subject to such storms and in the winter of 1886, three ice storms occurred in January and February, but this was exceptional. They were all accompanied by northeast winds with surface temperature at or a little above freezing, while slightly higher temperatures prevailed on Mount Washington.[2] Although meteorologists prefer to call such storms ice storms, locally near Philadelphia they are denominated sleet storms.[3] A brief résumé of the effects of the two ice storms above mentioned upon vegetation is of scientific import.

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[1] A cyclone, in the meteorologic sense, is an area of low pressure with inflowing spiral winds, seldom of destructive strength on land.

[2] Davis, W. M., *Elementary Meteorology,* p. 294, 1894.

The records show that on Sunday, February 16, 1902, snow began falling about two hours before midnight and by daybreak the ground was covered with a deep mantle which continued to increase steadily until Monday afternoon, February 17th, when eleven inches had fallen. Traffic on steam roads and trolley lines was seriously impeded, and pedestrians found it a laborious task to wend their way against the storm. At one o'clock Friday morning, February 21, 1902, sleet began to fall, which changed to rain and slush at nine o'clock. The rain continued during the day and well into the night with mean temperature of the day at 32 degrees.

Very few trees escaped damage from this storm. Trees that had withstood the storms of several centuries were uprooted, or had their larger branches snapped off by the weight of the ice. The large sycamore trees, *Platanus occidentalis,* L., in the yard of the Pennsylvania Hospital on Pine street were seriously damaged. These trees, noted for their large size and graceful branching, had their larger branches broken off as so many pipe-stems and the ground was littered with the fragments of branches that had stood the blasts of storms for over a century. Silver maple trees suffered most. At Haverford, Pa., where the storm reached its height, the avenues of trees along Lancaster Pike and intersecting roads were so badly destroyed that the highways were made impassable from the branches that had been torn from these trees. Fairmount Park presented a desolate appearance. On every hand, the ground was strewn with broken branches and splintered tree-trunks. From observations made by the writer, the forest and shade trees seemed to be injured in the following order: 1, silver maple most injured; 2, weeping willows; 3, Carolina poplars; 4, beeches; 5, elms; 6, hickories; 7, white oaks; 8, plane trees, especially the oriental species; 9, Kentucky coffee tree, almost not at all; 10, coniferous trees, pines, etc., not at all.

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[3] The *Standard Dictionary* (Twentieth Century Edition) defines sleet as "a mixture of snow or hail and rain, particularly a drizzling or driving partly frozen rain, or rain that freezes on the trees or ground."

At Horticultural Hall, Mr. John J. Prentzel recorded that a branch of an oriental plane tree, broken off encased in ice, weighed fifty-six pounds, and that after the ice had been melted off, it weighed nine ounces; a ratio of about 1 : 100. Dr. Swartzlander,[4] of Doylestown, Bucks County, Pa., weighed a twig with ice on and it weighed fifteen pounds. After melting the ice, the twig weighed nine ounces, a ratio of 1 : 26. Mr. H. H. Chapman[5] gives an account of the effect of this storm on Staten Island. The trees observed were elm, beech, tulip poplar and black oak, which had sound limbs broken that were four inches or more in diameter. White oak alone, according to Chapman, seemed to resist serious damage by the greater strength of its branches. To calculate the force which caused such destruction, twigs were cut transversely and diagrams made of the thickness of twig and ice incrustation. Calculating from the relative area in cross section, it was found that twigs one-eighth inch thick were carrying thirty to forty times their weight. In addition to this heavy weight, the trees were subjected to high winds, so that in many cases the crowns of trees were reduced fully 90 per cent.

The second storm of Saturday, December 13, 1902, gave the writer an opportunity to test the facts recorded above and also to ascertain additional facts of moment. Seventeen species of trees and shrubs growing in the Botanic Garden of the University of Pennsylvania, afforded material for study. The plan pursued was to cut off a limb, usually a lateral one, and weigh it surrounded by its sheath of ice. The ice was permitted to melt, the branches or twigs were then dried and carefully weighed. The difference in the weighings corresponds with the weight of the ice. A ratio was then calculated which expresses approximately the relativity of the force brought to bear upon the branches or twigs of the different trees. The accompanying table presents the results of the study:

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[4] *Forest Leaves,* VIII. October, 1902, p. 168.

[5] *Forestry and Irrigation,* VIII, 1902, p. 130; also *Experiment Station Record,* XIII, p. 1053.

No. Name of Plant.	Size of Part. Inches.	No. Lateral Branches	Weight with Ice. Grams.	Weight of Part. Grams.	Weight of Ice. Grams.	Ratio.
1—Rhododendron maximum	Blade 1.5 x 5	—	52	2	50	1:25
2—Rhododendron maximum	Blade 1.25 x 4.25	—	39.9	1	34.9	1:35
3—Tilia Americana	12	0	45	5	40	1:8
4—Populus nigra Italica ...	17	0	62	5	57	1:11
5—Liriodendron tulipifera..	10	3	50	9	41	1:4.5
6—Betula lutea	11	6	47	3	44	1:14
7—Maclura aurantiaca	36	2	439	42	397	1:9
8—Populus monilifera	22	9	97	14	83	1:6
9—Paulownia imperialis ...	22	0	360	65	295	1:4.5
10—Juniperus Virginiana ...	12	0	310	13	297	1:23
11—Pinus rigida	5.5	0	175	17	158	1:9
12—Picea alba	6	0	70	5	65	1:13
13—Picea nigra	15	0	598	65	533	1:8
14—Aesculus hippocastanum.	10	0	52	12.5	39.5	1:3
15—Acer dasycarpum	18	3	250	29	221	1:8
16—Platanus occidentalis ...	22	4	70	12	58	1:5
17—Ulmus Americana	27	6	320	24.5	295.5	1:12
18—Grass blade	2	—	5.5	.5	5	1:10

These ratios do not express the relation of the breaking weight, because in all cases the branches, some of which were considerably bent down by the storm, were removed before they were broken off. However, the damage during the first storm was undoubtedly due to the strong wind that was blowing at the time. The figures, however, give one some idea of the immense weight of ice and snow that a forest tree is capable of carrying under ordinary circumstances, and it is only when unusual storms come that the limit of strength of each particular species is reached and destruction occurs.

Phyllody in Nelumbo.

(*WITH PLATE XXVIII.*)

BY HENRY S. CONARD, PH. D.

A new dwarf lotus recently imported from Japan by Messrs. Dreer, of Philadelphia, shows remarkable reversions in the floral parts. The variety comes to us under the name of *Nelumbium Cihawan* (?), but is likely to be put upon the American market as *Nelumbo pygmaea alba plena*. It is easily cultivated. In a half barrel one may have five or six flowers at a time, and fifteen or twenty leaves. The latter are four to six or eight inches in diameter, of the usual peltate form, on petioles about eighteen inches high. Among the leaves or slightly above them are the creamy white "double" flowers, three to four inches across.

As is usual in Nelumbo the perianth leaves grade insensibly from the small triangular fibrous outer members to the large soft petals farther in. In this double form there is also a gradual transition from petal to stamen, as is so well known in roses, etc. These parts are all normally inserted around the stem at the base of the inverted cone-shaped carpellary receptacle.

The carpels have changed into large, hollow, leafy organs, open by a slit along one side and are deeply cucullate at the rounded upper end. Instead of being inserted in individual excavations of the receptacle, all of the three or four carpels are attached at the base of a single cavity. The cavity is about two-thirds as deep as the total height of the receptacle, and the rim is slightly scalloped, showing an indication of the separate cavities. The cup is very plainly an outgrowth from the upper part of the true receptacle. A number of well-known morphological principles are thus beautifully illustrated in one flower.

The close affinity of *Nelumbo* with the apocarpous Nymphæas (*N. cærulea, N. zanzibariensis, N. gigantea,* etc.), is also suggested. The stamens of *N. gigantea,* the most primitive member of the genus, closely resemble those of *Nelumbo* in the slender filament and curved anther. The receptacle, too, of *Nymphaea* is a cup-shaped organ, to the inside of which the backs of the carpels are fused, and on the outside the stamens are attached.

EXPLANATION OF PLATE XXVIII.

Nelumbo pygmæa alba plena, Hort. Flower and receptacle, natural size.

Observations on the Structure and Development of Epiphegus Virginiana*

(*FROM PLATES XXIX-XXXII*)

BY ETHEL COOKE, B. S., AND ADELINE F. SCHIVELY, PH. D.

Epiphegus Virginiana is a parasitic plant of Eastern America, to which frequent reference has been made in descriptive botanical works. It was first described by Linnæus in 1753, under the name *Orobanche Virginiana.* Later it was described under varying names, *Epiphegus, Leptamnion,* etc., by Nuttall, Rafinesque and others. No exact detailed comparative study of the plant was made until 1894, when Dr. Herman Schrenk published a paper entitled "Parasitism of *Epiphegus Virginiana,*" in the Proceedings of the American Microscopical Society.

Epiphegus Virginiana (Plate XXIX), commonly known as "Beech-drops," is a member of the dicotyledonous family Orobanchaceæ, allied by Warming to the Gesneraceæ, though the order is undoubtedly very close to the Scrophulariaceæ. It is parasitic on the roots of our common beech-tree, *Fagus Americana.* As a result of this parasitism, it has become extremely reduced and degraded. The mature plant is scarcely more than a mere inflorescence. *Epiphegus* illustrates well that general law of plant and animal parasitism, that reproduction becomes the chief function of the organism.

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* The above study was begun, and material in part collected, by Dr. Macfarlane in the summer of 1895. The work was continued by the second-named of the writers, but want of leisure prevented the investigation of microscopic details. At Dr. Macfarlane's suggestion the first-named of us undertook this, and brought it to the present stage of completion.—E. C. and A. F. S.

The degeneration of this plant is extreme and fundamental. Evidences of it are found in every structure possessed by the plant. Among its degraded characteristics are the loss of chlorophyll, the reduction of leaves to small scales, the rudimentary roots, the extremely simple dicotyledonous stem-structure, the cleistogamy of the more modified of the two flower-types, and the reduced undifferentiated embryo.

GENERAL DESCRIPTION AND MODE OF OCCURRENCE.

In searching for *Epiphegus Virginiana,* the varying character of the soil in which the plant flourishes is a source of constant surprise. Often in sterile regions, quite stony, under beeches, *Epiphegus* grows alone; then again, the soil may be a rich leaf mould, the home of many other species of plants. It is possible to find just as strong, tall, well-developed specimens of beech-drops under the former as well as under latter conditions.

It is curious to note also how the beech-drops often are confined to somewhat circumscribed areas. One may find a beech-tree around which numerous patches of *Epiphegus* are grouped; its neighbor may be without any. Then, again, the plants may be so scattered and at such a distance from the nearest beech, that one at first inclines to the supposition that the plant may be parasitic on other species than the beech.

The principal localities studied have been in the vicinity of Philadelphia. These were the eastern side of the Wissahickon, from Allen Lane to Thorp's Lane; the woods in the neighborhood of Crescentville; those near Lansdowne and Clifton, and a secluded corner in Woodlands Cemetery.

Epiphegus seems somewhat local in its occurrence. For example, there are many places on the Wissahickon where beeches were quite numerous, but no trace of the parasite could be found. This condition of affairs was also noticed at Eagle's Mere, also along the Rancocas Creek in New Jersey.

All early stages of *Epiphegus* were obtained more successfully in Woodlands Cemetery than elsewhere; possibly because the soil was loose in texture and there were comparatively few roots of plants other than the beech.

In the middle of June, the plants are found as minute white tubercles attached below to a slender rootlet of the beech-tree. They are quite smooth and spherical, and are as yet entirely lacking in any external differentiation. At this time they are from one-sixteenth inch to one-quarter inch in diameter.

In July they are found to be considerably enlarged and developed. They consist of a short, stout tuber-like structure, about an inch in length. It tapers smoothly above, and on its broadened basal portion gives off a number of short stiff curving roots, which are unbranched as yet. Above these roots, numerous overlapping scale-leaves cover the surface of the tuber. In the axils of the upper leaves appear the young developing flowers.

During the next few weeks a remarkably rapid growth occurs, so that in August the plant may be found in a fairly mature state. It may reach a height of eighteen inches, though it usually ranges from about six to twelve inches in length. In the mature plant, the stem is differentiated into two distinct regions—the tuber and the aerial stem. The tuber is the enlarged underground structure; it appears to be a central organ for the entire plant in its physiological activities as well as in its structural relations. It is through this tuber that the parasitic connection with the beech root is maintained. On its lower surface the tuber gives off the numerous short stiff branching roots. These roots do not extend far in any direction, but bend and turn about within a comparatively short radius. They branch freely, often a single root will branch three or four times. They are very stiff and rigid in texture. Scattered over the upper surface of it are numerous bracts or scale-leaves. These are small yellow-white, reddish or brown reduced structures.

Above the tuber rises the aerial stem, which is merely the axis of inflorescence. It rarely remains simple, more commonly it branches, and continues branching freely through most of its upward course. It shows a nearly constant torsion from left to right. This torsion is made evident in the grooves passing down below each of the flowers, and in the lines of darkened color occurring frequently along the stem. Both stem and branches are remarkably stiff, inflexible, unyielding structures. They are of a reddish or dull tawny brown color, and show streaks of darker brown, white and purple. The branches always arise in the axil of a bract. Some may arise separately on the tuber itself. Along the aerial stem and its branches appear the small bracts. They are arranged in a two-fifths spiral, sometimes in a three-eighths spiral; but in the upper part of stem and branches they seem to lose all regularity of position. In the upper part of the tuber these bracts are found, each subtending a small swelling or papilla. These swellings probably represent abortive branches. Bracts even occur on the lower part of the tuber between the outgrowing roots.

Throughout the greater length of stem and branches flowers occur in the axils of these bracts, a single flower in each axil. The total number of flowers developed on a single plant is strikingly large. These flowers display two distinct types—the cleistogamic or closed, and the chasmogamic or open type; there is frequently present a flower or flowers transitional between these two. The cleistogamic blooms are greatly in excess in point of numbers, they mature a much greater number of seeds, and are frequently the only kind of flower appearing on a plant. The chasmogamic flowers, when present, vary in number, as succeeding tables will show. When present, they occupy a limited region along the upper part of stem and branches, though they are scarcely ever found as the uppermost blooms.

Below them are often several "intermediate" flowers, showing some cleistogamic and some chasmogamic characteristics. Along all the remaining length of stem and branches appear the cleistogamic flowers. These are often borne far down on the stem, and may even be underground, as several writers have shown. Capsules are even matured on the tuber itself.

Until some time late in October, the inflorescence increases in height, or in number of axes or both. Up to the middle of August no flowers with expanded corollas are in bloom; after that time their production is quite constant, and continues late into October. Many plants never bear any chasmogamic flowers. In late summer and early fall, every stage of flower and fruit may be found in the woods.

Chasmogamic flowers rarely extend to the end of an axis. From upwards of four hundred axes examined only one instance of this was found. Beyond these, as has been stated, occur cleistogamic flowers.

There is apparently no rule which governs the production of chasmogamic and cleistogamic flowers. It is not a question of light, for in comparatively shady woods near Crescentville, many chasmogamic flowers were counted. In the sunny portions of Clifton woods, very luxuriant specimens from ten inches to two feet in height were seen. These likewise bore numerous chasmogamic flowers. In Woodlands Cemetery, a chasmogamic flower was never seen, nor were plants ordinarily more than six or seven inches high.

Observations concerning the relative number of chasmogamic flowers and also their relative position on the inflorescence were made upon plants mainly in the neighborhood of Clifton and Crescentville. In all instances, the lower third, sometimes half of the axis, produced cleistogamic forms. Above these, over an area that was extremely variable, occurred the chasmogamic flowers; the remaining portion of the axis was occupied by what appeared to be cleistogamic flowers; but some of these may possibly have been intermediate forms. In a very few cases chasmogamic and cleistogamic flowers were mixed in the same area of axis.

In order to get an idea of the rate of development and number of chasmogamic flowers appearing, specimens selected at random were studied closely. The method consisted in noting the number of axes of inflorescence. The number of chasmogamic flowers was counted, also the number of buds beyond these flowers. The buds included in this last series were never those which would evidently soon expand into chasmogamic flowers.

With some of the specimens further details were recorded. At the apex a red cord was tied and the plant allowed to develop. This marking was done on September 12 and plants were examined for final record during the first week in October. No axis showed at this time an addition to the number of chasmogamic flowers, but the number of cleistogamic buds was often greatly increased.

On the whole the percentage of chasmogamic flowers was much greater than was anticipated when the counting was begun. The accompanying table will give a fair idea of the observations. Records of many more than those given in Table I were made. One not included in this, because of space required, deserves a special reference. It was a quite tall inflorescence of twenty-seven axes; it bore one hundred and three chasmogamic flowers, most of which were found on eight axes, the other axes showing none. The exact number of cleistogamic was not ascertained, but was very large.

Number of axes of inflorescence.	Number of chasmogamic flowers.	Buds beyond chasmogamic flowers.	Additional branches of inflorescence.
8 a	11	6	1*
b	16	4	3†
c	14	6	2‡
d	16	6	2*
e	17	5	0
f	20	6	0
e	15	6	0
g	15	4	0
h	11	5	0
	135	48	

* All flowers were cleistogamic.

† Two branches each had six chasmogamic flowers and five buds beyond these. One branch had five chasmogamic flowers and five buds beyond these.

‡ One branch had cleistogamic only. One branch had chasmogamic only.

Number of axes of inflorescence.	Number of chasmogamic flowers.	Buds beyond chasmogamic flowers.	Additional small branches of axes.
6 a	0	0	0
b	0	0	0
c	9	5	0
d	0	0	0
e	0	0	0
f	0	0	0
	9	5	

Number of axes of inflorescence.	Number of chasmogamic flowers.	Buds beyond chasmogamic flowers.	Additional small branches of axes.
7 a	0	0	0
b	16	6	0
c	0	0	0
d	0	0	0
e	0	0	0
f	0	0	0
g	0	0	0
	16	6	

Number of axes of inflorescence.	Number of chasmogamic flowers.	Buds beyond chasmogamic flowers.	Additional branches of axes.
10 a	12	3	1*
b	18	3	4*
c	8	4	2*
d	5	4	2†
e	19	2	4‡
f	13	2	0
g	19	3	1§
h	20	7	0
i	18	3	0
j	20	3	0
	152	34	

* Flowers all cleistogamic.

† One branch had cleistogamic only. One branch had three chasmogamic and seven buds beyond these.

‡ Three branches had cleistogamic only. One branch had six chasmogamic and seven buds beyond these.

§ This branch had fourteen chasmogamic flowers and six buds beyond these.

Number of axes of inflorescence.	Number of chasmogamic flowers.	Buds beyond chasmogamic flowers.	Additional branches of axes.
11* a	5	5	0
b	13	3	0
c	0	0	0
d	11	3	0
e	8	4	0
f	3	7	0
g†	23	3	0
h‡	21	5	0
i§	24	3	0
j	7	4	0
k	20	4	0
	135	41	

* A very tall plant—over two feet.

Axes were tagged September 12th and examined first week in October, and had produced:

† Twelve more buds.

‡ Ten more buds.

§ Eleven more buds.

Number of axes of inflorescence.	Number of chasmogamic flowers.	Buds beyond chasmogamic flowers.	Additional branches of these.
6* a*	21	0	0
b†	13	0	0
c‡	10	0	0
d§	12	0	0
e‖	16	0	0
f¶	13	0	0
	85		

Axes were tagged September 12th and examined the first week in October, and had produced:

* Thirteen more buds.

† Four more buds.

‡ Three more buds.

§ Six more buds.

‖ Nine more buds.

¶ Eight more buds.

Number of axes of inflorescence.	Number of chasmogamic flowers.	Buds beyond chasmogamic flowers.	Additional branches of inflorescence.
14* a*	23	3	0
b	13	0	0
c†	15	2	0
d‡	21	0	0
e§	12	0	0
f	17	4	2‖
g¶	0	0	1‖
h	20	3	0
i**	17	3	0
j	0	0	0
	138	15	

Axes were tagged September 12th and examined first week in October, and had produced:

* Thirteen more buds.
† Thirteen more buds.
‡ Fourteen more buds.
§ Five more buds.
‖ Flowers were all cleistogamic.
¶ Twelve more buds.
** Fifteen more buds.

It was afterwards a matter of regret that all the cleistogamic flowers on the lower part of axis, as well as those on the upper part, had not been counted on the plants whose records were kept.

A series of observations that gave some idea of the approximate numerical relation of all flowers, was made during a succeeding season. A short résumé of the work of the two years is next given.

In September, 1899, plants of *Epiphegus* were examined in order to ascertain the number of chasmogamic flowers produced. An inflorescence shows ordinarily from fifty to seventy-five flowers, and quite often over one hundred. In several cases one hundred and forty were counted. In 1902 there was a marked decrease in the number of chasmogamic flowers borne. The character of the season may have had an influence. The summer of 1899 was warm and clear. In 1902 there was considerable rainfall; and August and September were very cool months.

In 1902 an attempt was made to study the comparative numbers of chasmogamic and cleistogamic flowers. There seems, however, to be the greatest possible variation; sometimes the two are nearly equal, but often there is a marked difference. The cleistogamic type predominates. The following tables present some results of these observations.

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
14 a	15	19*	3
b	17	5	1
c	11	0	—
d	18	5	2
e	19	2	2
f	16	4	4
g	16	7	3
h	12	9	2
i	13	8	2
j	17	5	4
k	14	6	4
l	14	8	2
m	10	0	—
n	16	0	—
	208	78	29

* One cleistogamic flower among these.

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
9 a	15	13	3
b	11	10	2
c	11	8	1
d	9	7*	1
e	17	0	—
f	12	6	2
g	10	6	4
h	16	0	—
i	17	0	—
	118	50	13

* Two cleistogamic among these.

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
6 a	23	14	2
b	14	6	1
c	10	0	—
d	19	0	—
e	16	5	3
f	20	0	0
	102	25	6

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
10 a	10	16	3
b	7	10	4
c	13	1	5
d	7	10	3
e	6	10	5
f	7	11	5
g	11	2	4
h	12	4	3
i	10	0	—
j	11	0	—
	94	64	32

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
13 a	14	8	—
b	10	7	4
c	12	7	5
d	12	7	6
e	12	6	5
f	12	8	4
g	10	8	4
h	12	8	4
i	13	4	3
j	16	0	—
k	13	2	4
l	12	0	—
m	8	defective	—
n	9	0	—
	165	65	39

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
7* a	2	12	2
b	13	6	4
c	13	8	3
d	12	11	2
e	14	5	6
f	12	9	3
g	15	3	5
	81	54	25

* And several very small ones.

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
8 a	14	16*	1
b	13	6	1
c	14	0	—
d	20	0	—
e	15	2	1
f	17	0	—
g	16	0	—
h	15	4	—
	124	28	3

* Four cleistogamic among these.

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
13 a	13	20	3
b	9	15	1
c	14	4	3
d	13	11	2
e	12	11	2
f	9	14	2
g	13	4	2
h	13	8	2
i	11	12	3
j	12	12	3
k	11	13	2
l	11	0	—
	141	124	25

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
12 a	31	5*	7
b	30	0	—
c	26	1	4
d	25	3	4
e	30	0	—
f	15	0	—
g	22	0	—
h	16	0	—
i	24	0	—
j	24	1	4
k	25	2	5
l	15	0	—
	283	12	24

* One cleistogamic among these.

Number of axes of inflorescence.	Number of cleistogamic flowers on lower part of axis.	Number of chasmogamic flowers.	Number of cleistogamic flowers beyond.
10 a	22	15	3
b	14	13	1
c	16	13	1
d*	15	12	2
e†	15	9	3
f	15	13	3
g	17	11	2
h	15	0	—
i‡	14	12	1
j	15	13	3
	158	111	19

* Three small branches, all cleistogamic.
† Two small branches, all cleistogamic.
‡ Three small branches, all cleistogamic.

The cleistogamic flower is quite small, rather flattened and zygomorphic, especially in its older state. It is raised on a short peduncle, and is set obliquely against the main axis. Just below the calyx are two small lateral tooth-like bracts and one large central one. The calyx is relatively of large size, is synsepalous and shows five tooth-like lobes along its upper margin. Above is set the small cap-like asymmetric corolla, fitting down within the lobes of the calyx. On its inner surface arise four small stamens, two being larger than the others. A well-developed ovary fills most of the flower cavity. Posteriorly arises a single style. It curves forward under the corolla cap, and bends down so that its stigma lies between the four anteriorly set stamens. Around the anterior base of the ovary a considerable nectary is developed. This probably represents the fifth stamen.

In the chasmogamic flower, the calyx and ovary are almost exactly similar to those above. The unlikeness lies in the structures of the distal region of the flower. The corolla is long and tubular, with open mouth. The style is very much longer here, each stamen is much better developed, as is also the nectary. Otherwise, the flowers are similar. The intermediate flowers have short tubular open corollas. The lengths of style and stamens are about midway between those of the cleistogamic and chasmogamic structures.

So these chasmogamic flowers are to be regarded as more primitive structures, persisting before the degrading influence of parasitism had affected the plant. The intermediate flowers show the course taken by increasing degeneration, in so modifying the flower as to produce the present functioning cleistogamic blooms.

HISTOLOGY OF THE MATURE STEM.

The aerial stem is triangular or tri-lobed in section, the lobes being separated by three deep grooves. These grooves are quite densely filled by hair outgrowths, though hairs are rare on the outer lobed portions of the stem. They are purely epidermal outgrowths, and vary much in size and in the number of constituting cells. Some are of eight cells, lying in a single row, and capped at the outer end by a transverse row of two or four cells. These multicellular hairs are the densest of all, containing rich cytoplasm and granular cell contents. Other hairs are long single cells, as large as the entire multicellular. These are empty and vacuolated, with a little faintly staining cytoplasm about the wall. It seems likely that these are worn-out multicellular hairs in which the cross walls have all or partly broken down. Some hairs show clear cuticular caps along their outer cell walls. These hairs are nearly all thin-walled, with a swollen turgid aspect, well adapted for an absorbing function. They must hold back and collect the water trickling down the grooves in the stem, and may even take up this water and its dissolved salts.

Stomata are frequently present along the stem epidermis. They show a very simple structure; there are two short swollen guard cells, with a slight orifice between. The entire stoma projects out considerably beyond the level of the stem surface. Similar simple stomata are abundant throughout the plant, being found on stem, bracts, on the corolla, even on the style, a quite rare occurrence in plants. This abundance of stomata is a usual accompaniment of parasitism. The parasite takes in an enormous quantity of sap from the host, as is evidenced by the rich deposit of starch through all its tissues. So a great excess of water must be brought into the plant, and a plentiful development of stomata is needed in order to transpire off this excess. Since leaves are present only in a rudimentary state, other organs are compelled to assist in producing these stomatic structures.

A noticeable fact in this connection is the rapidity with which the plant withers after being severed from its connection with the beech root. This is no doubt due to the excessive transpiration caused by the numerous stomata.

The stem epidermis consists of a single row of narrow cubical cells. A brown thickening is developed, especially on the outer wall, but to some extent on the other walls. The cortex cells are large and spherical, and are frequently separated by considerable spaces. Sometimes fine strands are seen connecting two cell walls, across such intervening spaces. The cells of the outer cortex are devoid of starch, but starch is abundantly present in the deep layers of the cortex and in the central pith region. Some starch grains are simple spheres, others are of a complex structure, consisting of a number of spherical segments fitting together. The bundles lie in a ring around the pith. Between them rays of the pith pass out to join the outer cortex, completely isolating each bundle, and so giving to the stem an aspect that is much looser and more primitive than that of the typical dicotyledonous stem. This state of affairs is probably only another of the degeneration effects due to parasitism.

In a section through a mature stem just above the tuber, the bundles form a quite regular narrow ring, its width being less than one-sixth the entire diameter of the stem. There were twenty-one separate bundles, varying greatly in size. There is an average size presented by about two-thirds of all the bundles. The remainder are mostly smaller, some being mere rudiments of bundles, less than one-twentieth the usual size. A few are larger than the average. Frequently two bundles are seen fused together.

An individual bundle is wedge-shaped in section, tapering inward toward the pith. The outer area is formed of hard bast cells, and is the most extensive cell area in the entire bundle. It consists of cells with extremely thick rounded cell walls and devoid of intercellular spaces. The greater extent of the cell wall stains scarcely at all, and is clear and refractive. The outermost layer stains deeply with saffranin, looking like a fine red line passing around the cell. It forms sharp angles at the cell intersections, while the inner margin of the cell wall is perfectly round and smooth.

Below these bast fibres lies the true phloem, nearly equaling them in amount. This region takes methyl green stain exclusively in saffranin anilin green double stain, and is characterized by protoplasmic, thin-walled delicate tissue. There are numerous large rounded phloem cells, and scattered among these are much smaller cells with a sharply angular outline. These cells invariably contain a dense, green-stained, balled-up mass. Their appearance is identically that of sieve-tubes in section, yet I have not been able to assure myself of the presence of sieve-plates in the longitudinal sections. It may be that degeneration has caused the loss of these structures, while the cells that possessed them remain.

The xylem cells lie next within, and are chiefly notable for their feeble development. The whole area of wood is very small, there being but two rows of cells present, sometimes three. These cells have thickened cell walls, stained with safranin, and include spiral tracheæ, annular and reticulated tracheids. This extreme reduction in xylem tissue is in accord with the colorless parasitic condition of the plant, and agrees with Wilson's and with Smith's observations on *Conopholis* and *Aphyllon.*

Within is the region of the internal phloem. This development was not noted in Schrenk's description of the stem bundles. It consists of but few cells, showing identically the structure of the outer phloem, with its small and large thin-walled cells. Rarely this area is as large as the outer phloem area. It is absent from some bundles. *Epiphegus* is therefore another plant that must be added to the rapidly increasing list of those showing bicollateral stem bundles.

Parenchyma cells lying next the bundles, both internal and lateral to them, show a tendency to develop thickening in their walls, and take on the safranin stain in the outer layers just as do the cells of the hard bast.

In sections made near the top of the stem, the general appearance is much the same as in sections lower down, except that the bundles are smaller and weaker-looking. There is considerable reduction in the relative amount of phloem here, though it is still much greater in amount than the wood. The internal phloem is here much reduced or may be quite absent. The triangular shape of the stem is more marked here than lower down, and hairs are more numerous.

This entire stem structure has evidently been greatly modified by the parasitic habit of the plant. The phloem is excessively developed, being the chief highway for the passage of food. For elaborated sap is taken directly from the beech and the phloem is the region fitted to convey such material. The xylem shows great reduction, and seems almost useless to the plant. For even the function of support has been transferred to the hard bast. It is this great development of bast fibres that gives the stem its stiff inelastic nature. It probably was in order to form an external protection to the enlarged phloem region, that such a great development of hard bast occurred. The whole bundle arrangement is evidently intended to allow the free passage of sap out into the stem at all places through its course, and to insure the abundant and widespread storage of food all along the stem.

THE TUBER.

Sections made through a large mature tuber present an extremely irregular and complicated aspect. Very striking is the great development through the parenchyma of thickened cells identical with the "hard bast" of the aerial stem bundles. These cells evidently are not a part of the true bundle system, and must originate in periblem rather than in plerome tissue.[1] The bundles show a most confused and irregular arrangement, running in all directions and planes apparently. The phloem of a bundle is greatly in excess of the xylem, and shows a tendency to spread out and lie in separate patches, while the xylem of each bundle seems always concentrated in a single area. Many of the bundles show an internal duplication with reversed order, phloem, xylem, xylem, phloem, succeeding each other from without inwards. In such a case, an area of thickened yellow stained cells often intervenes between the two xylem areas, and the whole may have resulted from fusion of two bundles. An internal phloem is almost always present, often in excess of the outer phloem mass.

A wide ring of the thickened, so-called "hard bast" cells lies below the narrow cortex, being quite regularly interrupted by thin-walled parenchyma cells passing in toward

[1] These rhizome sections were double-stained with Kleinenberg's hæmatoxylin and Bismarck brown, the thickening then taking on a yellow or brown stain.

the pith. Frequently almost the entire central core of the tuber has cells showing this yellow-stained thickening, and sends out great radiating arms of such tissue through the bundle region, forming a star-like pattern.

The xylem occurs in small areas of thickened cells, spiral and reticulated tracheids, no long trachea appearing. Scattered in among these tracheids are the peculiar large protoplasmic cells that are associated in parasitic connection with the host, as will be shown later.

The phloem cells stream outward and inward from the xylem in irregular masses and long radiating arms. The cells are small, angular and of two sizes. They stain deeply, seeming to be densely protoplasmic The smaller cells, the degenerate sieve-tubes, probably possess a stained nucleus lying next the side wall. In longitudinal section the phloem is seen to consist of remarkably small cells. They are both short, and extremely narrow and fine. They sometimes taper to a point, sometimes meet with flattened ends, which have each a thickened cap-like structure. This may be the remains of the sieve-passage between cells. External to each bundle there is usually a considerable development of the thickened brown-stained fibrous tissue or "hard bast."

The fundamental tissue of the tuber shows two well-marked regions. First, there is an area lying external to the outer bundles and the circle of hard bast. This area shows large circular cells with somewhat thickened walls, all taking the brown stain. The cells are entirely empty, have neither nucleus, cytoplasm nor starch. The outermost cells are stretched out, often split apart and in process of sloughing off. They have deeply stained thickened walls, and frequently show numerous large, oily-looking globules, a tannin precipitate probably. The inner area of the parenchyma lies within the hard-bast ring, and is sharply distinguished from the outer one in that its cells take on hæmatoxylin stain. The cells are large, thin-walled, irregular in outline; they contain a pale brown-stained nucleus and nucleolus, and have remains of cytoplasm. They are nearly filled with compound starch grains. The central pith cells also contain a little starch, but the outer pith cells, near the bundles, are densely packed with starch deposits.

HISTOLOGY OF THE MATURE ROOT.

The root is very nearly circular in cross-section. There is a single epidermal layer of cubical thin-walled cells. These walls are thinner than those of the parenchyma cells within, and have a brownish-yellow color in the fresh material. They contain a brown dense elliptical nucleus lying against the inner wall. These epidermal cells never have a flat external surface in the fresh state. The external wall either bulges outward or is depressed and curves inward. This fact, together with the thinness of the walls, seems to indicate an absorbent function in these cells.

The parenchyma is of large round thick-walled cells, densely filled with starch grains. These cells do not taper longitudinally, but flatten against each other. There are strong indications of pore connections through their common cell walls, and also a very evident lamellation. A large nucleus is frequently seen against the glistening yellow cell wall, while considerable protoplasm and a nucleolus are usually evident. Starch grains are abundant and are mostly single or double, the complex forms being rarely found in the stem. Some show an irregular, semi-dissolved outline. Occasional parenchyma cells show a clear matrix, in which lie, in a parallel direction, numerous shining crimson needle-crystals. They are slender spindle-shaped forms, with thick centre and pointed ends, about one-third the length of the cell. Such cells occur in groups of two or three and contain no starch.

There is a small central bundle, not sharply separated from the cortex, as an endodermis is absent. Across it passes a central band of wood, consisting of a few spiral tracheæ and some reticulated tracheids. On both sides of these cells are present some small dense phloem cells. The parenchyma cells lying around the bundle for some distance show very greatly thickened, shining cell walls. The bundle is not sufficiently well developed to afford much support. So it must be these thickened parenchyma cells that give the stiffness and rigidity to the grapplers.

There has been noted a great deal of variation in the production of these roots. Plants growing in a soft rich soil develop a great number of them. Those already supported by a sandy or stony soil develop very few. This seems to indicate that their chief function is one of support for the parasite. But the structure of the epidermis, and the richly protoplasmic, starch-filled parenchyma, render it probable that they also possess absorbent properties. And absorption would also be greater in the rich humus than in sand.

The bundle can be seen passing off from a large bundle in the tuber. It is quite broad here, but narrows steadily as it passes out. It extends clear to the extremity of the root. No trace of a root-cap has been found in any plant I studied, though it has been very carefully looked for. Yet this grappler must be considered a true root, as will be shown later. The root-cap accordingly has been lost by degeneration, as various investigators have already shown for other degraded parasites.

THE DEVELOPMENT OF THE ROOT.

In very young tubers no roots have appeared on the surface, but their internal formation is then evident. They are first seen as a group of embryonic cells pushing out from one of the undifferentiated central bundles of the tuber. This group of cells advances through the tissues of the tuber, leaving behind it a gradually forming bundle, that becomes the central bundle of the root. By the time this advancing group of cells has reached the circumference of the tuber, it has formed a distinct row of epidermal cells along its front. A root sectioned just before its emergence from the tuber was seen pushing out the tissue of the tuber as a great swelling (Plate XXX, Fig. 1). There were but two more rows of cells left to be penetrated, and these were just being split off at one end by the pressure. A distinct space intervened between the newly formed epidermis of the root-tip and the double row of cells lying beyond.

In somewhat older tubers, where the roots have attained to some length, there is still a considerable area of embryonic cells at the tip of the root. This area is cone-shaped, its base lying across the root-tip. It consists of small cells, with a large nucleus and densely stained cytoplasm.

In none of these earliest stages is there any trace of the formation of a root-cap. This endogenous origin of the structures called grapplers by Schrenk, establishes their morphological nature as true roots, though undoubtedly they are very degraded ones.

Comparative Histology of the Flowers.

Many interesting facts are revealed by a detailed comparison of the evident or chasmogamic and the cleistogamic flowers. They are clearly constructed on the same general plan, but are modified so as to function differently. One flower represents the original primitive condition in structure, though it shows a great loss in vigor in the present state of the plant. The other flower illustrates the reduction and degradation that may accompany parasitism. The cleistogamic flower is an admirable example of structural modification to suit definite function; it also shows to what an extreme the reduction of flower parts can be carried, while there is still abundant production of seed.

The Calyx.—This is identical in structure in the two flower types. It is synsepalous and shows five evident lobes. Five bundles traverse the calyx, one ending in each lobe. These bundles are relatively well developed. There is a core of long slender spiral tracheae extending out farthest in the lobes. On both sides of this occur short stout spiral tracheids, set irregularly and discontinuously along the bundle. A few small phloem cells lie to one side. The tissue cells are much larger than the bundle cells. They fit together in all kinds of positions, except near the bundles, where they arrange themselves quite regularly.

One-celled, rarely two-celled hairs fringe the edges of the lobes. Below, across the base of the lobes, there extends a band of two or three-celled hairs, longer than the upper hairs. All of these hairs are on the outer surface of the calyx; none are present on the inner surface. They have a swollen granular appearance.

The Corolla.—In the chasmogamic flower (Fig. 3) the corolla consists of cells longitudinally elongated, and is of four or three cells in thickness. The outer layer of cells is quite regular and even, constituting an epidermis. At the base, where the corolla passes under the calyx, it thickens greatly, being many times thicker than either the calyx or the upper corolla region, and consisting of two cell layers. Five vascular bundles pass through its length and end in the five corolla lobes. They are similar to the bundles of the calyx.

There are two areas of hair development, both on the inner surface. One area forms a hairy belt along the outer margins of the corolla lobes. These are short, mostly two-celled hairs, and often so bend on themselves as to have a hooked appearance. They are greatly swollen and turgid-looking. The other area is a ring extending round the inner surface at about the region where the stamens are inserted. These hairs are much longer than those above. They consist of about three narrow elongated cells. At the free end of each hair there is often a flat, flange-like expansion.

The cleistogamic corolla is greatly reduced in size. It becomes an apparently closed asymmetric cap-like structure that is lifted off by the maturing ovary. The bundles are also reduced. A small fifth one may appear, but usually there are but four bundles. Two of these are fairly large, and show both spiral tracheae in three or four rows, and tracheids. They occur about one-third of the circumference of the corolla apart, and arise on the anterior short curve of the cap-like corolla. In that position they can best support the corolla. The two small bundles alternate with these large ones. They apparently contain but a single spiral trachea, that extends clear to the end of the corolla. The larger bundles end some distance below. Stomata have been found on the corolla.

This corolla is from four to six cells in thickness. The outer layer is of large regular close-set cells, with a considerable amount of thickening developed on their outer walls. A protective epidermis is thus supplied to it. Longitudinal sections reveal that the corolla is not in strict morphological sense a cleistogamic one, but has a distinct though small mouth (Fig. 4). This opening occurs where the anterior straight, or slightly concave and convex surfaces of the corolla meet. The convex surface develops a disc of greatly thickened cells that overlaps the concave side, leaving a distinct mouth. This mouth is entirely closed by the numerous small hairs crowding into it from within the corolla. These hairs are of one to two round swollen cells. They arise in great numbers on the inner surface of the corolla all about this mouth. Also a group of hairs appears on the outer surface of the corolla, tip, just where it is overlapped by the other surface. These hairs help greatly in filling up this mouth. The corolla may then be called physiologically but not morphologically cleistogamic. There is a slight development of long hairs about the region where the stamens arise.

The hairs closing the corolla's mouth are evidently intended to keep out intruding insects. Probably the numerous hairs developed on the chasmogamic flowers have also a definite purpose with regard to insect visitors. But just what this purpose is has not been discovered.

The Androecium.—There are four stamens inserted on the corolla and one-third from its base. This is true of both cleistogamic and evident flowers. But in the cleistogamic flower the stamens show great reduction in size and an actual increase in the production of micro-spores (Fig. 4).

In the chasmogamic flower the filament shows a large central bundle. At the base, both phloem and xylem are well developed. But the phloem constantly decreases in amount toward the anther region. Hairs develop across the inner face of the filament, just above its origin on the corolla. They are nearly identical with the hairs of the corresponding area on the corolla, being very long, narrow and multicellular.

The anther consists of two lobes that hang dependent from the upper tip of the filament. They are remarkable in being so distinctly separated from each other. They meet in the upper portion, but diverge widely below, being found on opposite sides of the filament.

There is a great development of hairs even on these anther lobes. Each hair is extremely long, slender, unicellular and has tapering ends that often hook downward. A great deal of thickening is developed in the cells of these anther walls, of a clear, shining, refractive appearance. In section, this thickening is seen to be deposited quite evenly about the walls of the two or three rows of cells that form the anther sac. But in the late stage of the anther, this thickening is loosened out from the cells and split up in various ways, so that bars and angles and even squares of glistening thickening substance are seen lying over each other in the greatest confusion. This state of affairs was always noted when dehiscence had occurred, so it was probably a result of dehiscence. The line of dehiscence is a vertical one, passing from top to bottom of the anther wall.

In the cleistogamic flower, the filament shows the same structure as the evident one, but it is much smaller. The hairs developed on its lower portion are greatly reduced both in number and in size. The anther lobes are very much smaller also, but differ scarcely at all in structure. The only noticeable difference is in the greatly reduced development of hairs on these lobes. None at all are present on the upper and lateral surfaces; a few are scattered about on the lower surface.

The great reduction in the development of hairs within the cleistogamic corolla is probably due to the fact that definite insects are excluded, and no provision has to be made to ensure their aid in fertilizing the ovules.

There is no observable difference between the pollen grains of the two flowers. And although the anther cavities are much larger in the evident flowers, yet the grains lie much nearer together in the cleistogamic stamens. By actual counting of the numbers of pollen grains found in many sections of anther cavities in both kinds of flowers, it was found that the average number was greater in the case of the cleistogamic flower.

The Pistil.—The structure of the pistil is nearly identical in the two flowers, though the styles differ greatly in length (Figs. 3 and 4).

The ovary is more narrow and high in the chasmogamic flower, and is shorter and broader in the cleistogamic one. Otherwise the following description applies to both. The ovary is superior, one-celled, with four parietal placentas running vertically through its walls, and projecting inward from them. Each placenta is traversed by three bundles, a large central one and a smaller one on each side. There are usually five bundles rising in the ovarian wall, there being one behind each placenta always. A definite epidermis of small regular cells lines the cavity of the ovary. It passes out continuously along the funiculi, where the ovules are given off, so that the placentar and funicular tissue are directly continuous.

In immature ovaries, there is a considerable empty space in the centre of the cavity. In later stages the ovules have so enlarged as to entirely fill the ovary. They lie in a uniform structureless, faintly stained matrix. Starch is quite abundant in the walls of the young ovary, and gradually increases in amount in older ovaries. But in the mature capsule there is relatively little present. It has probably been used up in the growth of the ovules.

As the ovules grow and fill up the cavity, a considerable tension is brought to bear on the walls of the ovary. The outer cells of this wall assume a stretched-out, flattened appearance, and frequently are torn completely away. This tension produces the vertical split that occurs lengthwise across the summit of the ovary. In a still later stage, the entire tissue of the ovarian wall has assumed a stretched-out appearance. But the inner cells of the wall, on each side of the longitudinal split across the capsule, have enormously enlarged and become crowded with starch grains. Very little starch is now present elsewhere in the ovary. Now the action of the valves in dehiscing is to pull apart and curl their edges outward and downward. And the downward pull of the stretched ovarian wall, together with the outward growth of the two swollen cushions along the split edges, would produce this rolling of the valves outward and downward.

The Nectary.—This is present alike in the cleistogamic and the evident flowers. It is generally similar in each, but is somewhat smaller in the cleistogamic flower, especially so in somewhat later stages. It appears as a swelling on one side of the ovary, antero-laterally in position just above the base (Fig. 5). Neither its depth nor its thickness is very great, but it has considerable length, running half-way round the ovary in chasmogamic flowers. It is not so long on the cleistogamic ovary.

The nectary consists of a kind of tissue, strikingly unlike the surrounding cells in general appearance. It consists of rather small, spherical thin-walled cells, showing no intercellular spaces. All of these cells are well filled with deeply stained protoplasm, and have a very large and dense nucleus and nucleolus. Starch grains are present in considerable quantity.

This nectary may probably be regarded as the remaining trace of the fifth stamen that should be present to preserve agreement of number throughout the flower. The presence of this nectary is also evidence that this plant was once a typically insect-pollinated form. It is rather remarkable that it should be so well developed in the cleistogamic flowers of the present time, where it is entirely useless. This persistence of the nectary, and also the production of the primitive chasmogamic flowers, seem to indicate that parasitism in this plant must perhaps not have been of so very ancient date. At any rate, modifications due to parasitism are still occurring, and the plant is at present in a somewhat transitional state. This is especially indicated by the production or non-production of chasmogamic flowers, by the variable development of roots and by the evolution of two types of floral structure that are united by intermediate stages.

The style and stigma are identical structures in the two flowers, except for differences in size. The style is elliptical in section. Two vascular bundles traverse its length. These are well developed, and show phloem cells, also numerous spiral tracheæ. Toward the stigma these elements loosen and spread apart noticeably. Simple two-celled stomata are numerous along the entire length of the style, but are present in greatest number midway between the ends.

The stigmatic surface has the aspect of a broad convex cap set on the end of the style. It is thickly covered with short, round swollen unicellular hairs.

There is a marked horizontal division through the style. In all cases there is evident a thinned-out, broken region across the style midway between the two bundles. In the upper part this may often increase to a considerable cavity, so that in effect there are two styles present. If styles are forcibly compressed under a cover-glass, they split readily in two along their entire length. In some cases the stigma also shows this bifid nature. In one flower that was sectioned, the style forked in two in the middle of its course, and each of these two styles bore its own separate stigma.

It is interesting to observe the special arrangements of style and stamens so as to provide for cleistogamy. The small cavity within the cap-like corolla is nearly filled up by the four stamens and the style. There is little free space left. The style rises at the extreme posterior region, passes upward and curves forward (Fig. 4), close under the broader arch of the cap-like corolla. Anterior to the style rise the four stamens, two on each lateral wall, curving inward toward the stigma. In an older cleistogamic flower, the style has curved more strongly forward and then downward again. The broad stigmatic surface now lies facing forward and somewhat downward. The filaments have pulled the anthers downward by a peculiar twisting and bending on themselves. So now the two anther lobes of each stamen are seen closely appressed against the stigmatic surface. The stigma lies with two anthers pressed against it on both sides. Great numbers of pollen tubes are seen passing from the anthers over into the stigma. Such a close attachment is formed that it is impossible to tear the anther away from the style without completely destroying the tissues of both structures.

Evidently the peculiar form of the corolla, resembling in shape a liberty cap as nearly as anything, depends upon the positions of pistil and stamens. The broad convex surface of the cap is the surface overlying the curving style. The shorter face of the cap indicates the curving planes along which the stamens are ranged.

This peculiar arrangement of style and stamens supplies the explanation for the greater seed production in one valve of the capsule, curious as this may seem. The more anterior and somewhat right-hand of the two valves always contains considerably more seed than does the posterior valve, 950 to 770 being a fairly average ratio. The style is divided in two regions, corresponding to these two valves. The posterior stylar half lies above, next the exterior of the corolla, when it has curved over to reach the stamens. So the anthers lie adjacent to the lower half of the style, and send their pollen tubes in here. Naturally, therefore, more pollen tubes pass down the anterior region of the style, and more ovules are therefore fertilized in the anterior valve of the capsule.

Fertilization in the chasmogamic flowers is probably by means of insects. The nectary and peculiar arrangements of the numerous hairs seem to indicate this. Protogyny is evidently the rule here. On a number of plants observed in early September, the lower and older evident flowers showed a brown, shrunken, dried-up pistil, while the anthers were just about mature, some having dehisced, but not yet shed their pollen. The upper and younger chasmogamic flowers showed a mature pistil, with sticky stigma bearing numerous attached pollen grains. The end of the style curves over and downward so that insects must brush by it in going to the nectary. In these flowers the anthers were decidedly immature in appearance. So protogyny is the existing condition.

THE DEVELOPMENT OF THE OVULE.

The following observations were made entirely from cleistogamic flowers.

In very young buds, the ovary is seen to be filled with pale, faintly staining cells. Through these cells pass vertical double rows of very deeply stained cells. These are the earliest beginnings of the ovules. The cells are rectangular, with dense cytoplasm and a very large nucleus. The nuclear cavity is clear and almost unstained. In it are seen great irregular lumps of chromatin massed along fine linin threads. In the centre is a large spherical densely stained nucleolus (Fig. 6).

In somewhat older flowers the ovules have assumed their definite form. They are attached to the placenta by long curving funiculi. The funiculus consists of rows of elongated faintly stained cells, with a large pale nucleus and very small nucleolus. The seed-coats have appeared. At first a single one is present, composed of a double row of rectangular nucleated cells, surrounding the embryo sac. Other ovules show both seed-coats, being surrounded by four rows of cells. Their anatropal state is now evident, the micropyle occurring next the funiculus.

Down the centre of the ovule extends the embryo sac. It is comparatively short at first, but later becomes almost as long as the ovule itself. It is a great elongated cell, bounded by a definite wall and filled with densely stained cytoplasm. In the youngest stage a single very large nucleus is present. It possesses a relatively enormous nucleolus that is spherical, and very densely stained chromatin is seen in masses streaming through the clear nuclear cavity. Later this nucleus divides in two, and the two nuclei move apart toward the poles of the cell (Plate XXXI, Fig. 7). When they lie at the poles, each is seen surrounded by an aggregation of cytoplasm, while a considerable vacuole has appeared in the middle of the elongating embryo sac, or macrospore cell. A shrunken thread-like protoplasmic connection is retained between the two separated masses (Fig. 8).

Division of each of these two cells next occurs, the axes of the two spindles being perpendicular to each other. The upper or micropylar spindle (Fig. 9) lies at right angles to the long axis of the ovule. The lower distal spindle lies parallel to the axis of the ovule. These spindles are long and tapering in outline, apparently ending in pointed apices. So in the four-celled stage, the two pairs of nuclei lie at right angles to each other. Later the two upper nuclei turn about, so that they lie one above the other, just as does the lower pair. The sac elongates steadily, and the connecting thread of protoplasm between the two poles becomes considerably longer. There seems to be no increase in the total amount of cytoplasm present.

Next, division occurs in these four cells. Similar pointed spindles form, on which lie many small stout rod-like chromosomes. The two spindles at the upper end show exactly the same relation to each other as did the two spindles of the preceding stage. Division of the two lower cells was not observed, but it is supposed the spindles lie in the same relations as in the upper region, since the relative positions of the nuclei after division indicate it.

In the mature embryo sac, the cytoplasm is aggregated in three distinct masses. It narrows toward the lower free end of the ovule, and is broadest just below the micropylar end. The eight typical nuclei are now present. The two synergidal nuclei lie side by side just below the micropyle. A little farther down is the egg nucleus, larger and more deeply stained than the synergids. Below is a sharp transverse break in the cytoplasm. Right under this lie the two fusion nuclei. They are seen to be approaching each other, though lying some distance apart. Later they lie side by side (Fig. 10), closely appressed and flattened against each other. They are the largest, densest nuclei in the entire embryo sac. They have a large amount of deeply stained chromatin, and a very large, dense spherical nucleolus. At a considerably later stage they are seen to have fused to form the single large endosperm nucleus.

Below, in the same mass of cytoplasm that contains the endosperm nucleus, extends the long central vacuole before noted; it is surrounded by narrow protoplasmic walls. Another complete transverse split in the cytoplasm occurs (Figs. 10, 11). In the lower cytoplasmic mass lie the three antipodal cells, the lower two lying side by side, and having the appearance of being separated by a faint cell wall. They are rather smaller and paler than any of the other nuclei in the embryo sac or macrospore cavity.

In the next older stage, the ovule is seen increased much in length, but little wider. Starch is appearing in the walls of the outer seed-coat. At the micropylar end, a long, narrow neck is left between the seed-coats. Below this lie the remains of the old embryo sac, still showing the two synergidæ above the egg nucleus. A break occurs just below, and then is seen a vertical row of six to eight cells, larger below, smallest next the egg. This is the precocious albumen or endosperm, formed by division of the endosperm nucleus prior to fertilization. These cells are densely protoplasmic, and show a fairly large nucleus and nucleolus. They are separated from the cells of the seed-coats by a thick stained membrane.

In ovules observed at about the stage of fertilization, a great increase has taken place in the amount of endosperm cells. Three or four long rows of cells are present, formed by divisions which have been observed occurring in both directions. The spindles formed are remarkably broad and barrel-shaped in form. These endosperm cells have grown up around and above the egg cell, and form a structure resembling an archigonial neck below the micropyle, having pushed apart the seed-coats. Down this neck the pollen tube is seen passing to the egg cell. It is faintly stained and almost transparent, containing little cytoplasm. The process of fertilization was not observed. After the egg has been fertilized, there is always observed a second degenerating nucleus lying in the pollen tube just above the egg cell. This is probably the second sperm nucleus that in many plants has been found to unite with the endosperm nucleus. But here, the endosperm nucleus has already divided and formed the precocious albumen, so this sperm nucleus would necessarily be non-functional.

The first cleavage wall divides the egg in horizontal direction. The upper cell is smaller, both in the size of the nucleus and the amount of cytoplasm (Fig. 12). The lower cell has a much larger mass of dense cytoplasm. Its nucleus is very large, and contains a large dense nucleolus, while strands of chromatin pass through its clear cavity. It is this cell that later gives rise to the main mass of the embryo. The upper cell only forms a small, suspensor-like structure of four or five cells. During this two-celled stage of the embryo, there is little differentiation between embryo cells and albumen cells. But as development progresses, the difference constantly increases. The embryo cells become relatively much smaller and denser-looking. The albumen cells enlarge greatly, stain less and less densely and finally develop a great quantity of starch. They also continue to increase somewhat in number by division.

The lower embryo cell then divides in a plane perpendicular to the first division, giving two nuclei that lie side by side (Fig. 13) and later separate by a vertical cell wall. Then these two cells each divide in the same horizontal plane as the second cleavage, but in a direction on this plane at right angles with the direction of the second cleavage. The spindle observed here possessed sharply pointed ends (Fig. 14). Next these four cells all divide vertically, giving an eight-celled stage consisting of two tiers of four cells each. Subsequently each of these eight cells divides unequally, so that the axis of the spindle must lie in a radius from the centre of the mass. After these cells have formed, the appearance of a cross-section is as if four oblique radii were passing out from the centre of a circle. On the line of each radius lie two cells, the inner one smaller, the outer one much larger. Later an oblique division wall appears between each of these two cells. So these four division walls form a diamond, within which lie four small cells, and outside of which lie four much larger cells (Plate XXXII, Fig. 16).

After this sixteen-cell stage further regularity in division cannot be traced, though division proceeds rapidly. The eight large outer cells frequently divide in a plane perpendicular to the preceding division, so that two smaller cells lie side by side beyond the oblique wall. There is no indication of any differentiation into special regions such as is usually shown in normal embryos. The whole mass remains spherical and undifferentiated.

The upper cell of the two-celled stage has proceeded slowly with its division, while the lower cell has been thus active. It divides twice, to form two equal cells above, and a small flattened lenticular cell below. The middle of these three cells then divides in two in the other direction, so that there appear two smaller cells lying side by side (Fig. 16). These four cells form a kind of elongated neck rising above the spherical mass below. In the latest stages observed, I have never seen any further differentiation in this structure, which is evidently a rather degraded suspensor.

In the mature ovule or seed, that is ready for discharge, the embryo appears one-third the distance below the micropyle. The albumen cells have increased to eight or more rows. They have enlarged greatly, especially the outer cells, and have become fainter in staining capacity. The cytoplasm is reduced in amount, the nucleus is rather pale and faint-looking. These cells are cubical usually, and well filled with starch grains. A considerable cellulose thickening develops along the regular outer row of albumen cells, and forms some distance down thin division walls. Of the seed-coats, only the outer row of cells is now functional. The remaining cells lie in shrunken narrow strands outside the albumen. This outermost row of cells is enormously enlarged. Each contains little cytoplasm, a pale nucleus and vacuolated nucleolus, and numerous large starch grains. There is a great development of a dense clear shining wall-thickening, that looks like cork. In surface view, this appears like a lattice-work, of long parallel bands joined by occasional short cross-bars. In section, this thickening is seen to develop entirely in the side walls of the cells, as a broadly lenticular mass. The outer and inner walls are quite unthickened.

DEVELOPMENT OF THE POLLEN GRAIN.

In the anthers of the very young cleistogamic buds, the following stages of microspore development were noted. The anther wall shows two or three rows of embryonic cell tissue, with unthickened walls. Within, the sporangial cavities are completely filled by regular rows of large, nearly cubical "spore-mother" cells. They are filled with cytoplasm that reveals a distinct network structure of stained nodes and pale connecting threads. Each cell has a large nucleus, and its clear cavity is scantily filled with granules of chromatin lying on slender linin threads. There is a large spherical pale nucleolus that appears entirely unstained (Fig. 17a). This nucleolus is strikingly different from any other observed throughout the entire plant. The macrospore cells, on the other hand, show the deepest stained nucleoli present in the plant.

Later, this mother-spore nucleus goes into a distinct synapsis state (Figs. 17 b-d). The nucleolus grows even fainter, and the chromatin gathers about it, passing in along the linin threads that are seen streaming from all parts of the cytoplasm to the nucleolus. Finally, the nucleolus is entirely covered up by the great crowded, densely staining, balled-up chromatin mass, while the nuclear cavity around is perfectly clear-looking and unstained. It is a typical synapsis stage. Some time later this nucleus goes into a typical resting stage, and then shows chromatin granules on a fine network of linin threads, in a clear nuclear cavity. The nucleolus has entirely disappeared (Fig. 17 e). Finally, the spore mother cell undergoes its first division. A broad, barrel-shaped spindle is formed, very unlike the slender pointed spindles of the macrospore divisions (Fig. 17 f). Then two large nuclei lie side by side in the spore protoplasm (Fig. 17 g). These divide again, and the four smaller nuclei lie close together in the space, their contact walls being flattened against each other (Fig. 17 h-i). In slightly older anthers, these tetrads appear as four separate balled-up nucleated masses of cytoplasm, lying in a clear shining mucilaginous-looking mass. At this stage a great number of disintegrating cells are lying around next the anther wall, a degenerating tapetum probably.

The mature pollen grain shows a double layer of clear refractive thickening round its wall. Within is densely stained protoplasm. It is invariably divided into two distinct masses, separated by a considerable space (Fig. 17 k). One mass is very small relatively, and lies flattened against the wall. It contains a small clear elliptical nucleus, with a large dense nucleolus, and chromatin scattered along the nuclear membrane. The larger protoplasmic mass shows two slight lobings. A clear vacuole fills most of one lobe. The other contains a large nucleus, spherical in outline, with considerable chromatin and a large dense nucleolus. This is probably to be regarded as the prothallial nucleus, while the small flattened nucleus is the generative nucleus.

Somewhat later, the pollen tube is seen emerging. The spore wall looks thin and eaten away at one point, then a bulging of the protoplasm occurs here. The tube pushes out into the bulging here, the small nucleus lying against its side wall. The large nucleus is seen remaining in the pollen cell, and probably disintegrates there, as it is not observed later.

The pollen tube grows across and enters the stigma, pushing its way between the cells and down the style. Two nuclei now appear in it, undoubtedly resulting from a division of the generative nucleus, and forming the two sperm nuclei that are later noted in the macrospore cavity.

DIFFICULTIES OF GERMINATION.

It is surmised that for successful germination, the seed of *Epiphegus* requires contiguity with the beech-root. The seed falls upon the soil and is carried probably but a little distance below the surface. At least this would be inferred from experience in gathering the tubercles. Thus the seed lies loose in the soil, awaiting the favorable contact of the beech-root. Now such variation in the size and time of appearance of these plants is shown, that granted the seed's ability to retain vitality, the germination may occur when the new roots of beech reach their vicinity, even though this should be early in the next season. No other hypothesis will explain the constant succession of young plants well into the summer.

Several attempts to study the germination have been made, but as yet these have proved unsuccessful. Many factors combine to render this a difficult problem. The extreme minuteness of the seeds, the small proportion of these which develop into plants, the apparent necessity for chemotactic contact with the beech-root, and possibly some soil peculiarity, all combine to make successful study somewhat troublesome.

The Development of the Young Plant.

A young tuber about one-sixteenth inch in diameter has an irregularly elliptical outline, being longest in a vertical direction. It has a one-celled epidermis of cubical, nucleated cells, with slightly thickened walls. The cortex is of large thin-walled cells, well filled already with starch. A few bundle-masses stream irregularly through the cortex, in every direction, near the base of the tuber. Several of the larger bundle-masses lie about in the centre of the tuber. These bundles are of densely stained embryonic cells, with scarcely any thickening developed. They are still in a quite primitive, undifferentiated state.

No roots have emerged as yet. One only may be seen forming within the tuber. But the group of embryonic cells is as yet in a relatively deep portion of the cortex. There is in the whole tuber only this single root-trace as yet.

Such a plant is one that establishes a lateral connection with the beech-root, so to speak. That is, the attachment to the beech-root occurs some distance above the base, on the side walls of the young *Epiphegus.* Others form the connection directly through their base. Evidently the ovules giving rise to the latter rested above the beech-root and germinated in that position. The seeds must in all probability lie beside the beech-root to form the lateral connection. Two young tubers attached in the same plane on a beech-root, one on either side, have been observed. Mature plants sometimes show the same condition.

In the one here described the connection was lateral. Near the point of connection the epidermal cells of *Epiphegus* take on a peculiar darkly colored appearance. At this level the several bundle-masses in the tuber seem to stream across in nearly parallel direction, converging toward the haustorial connection.

In most cases of parasitism the connection between the two plants is formed by the parasite, that pushes its suckers into the tissue of the host. But in *Epiphegus,* all indications point to the conclusion that the host sends the "haustorium" *into* the parasite (Plate XXX, Fig. 2). The appearance in sections through this region is of a number of arms growing from the beech-root into the parasite. The peculiar tissue composing these arms is totally unlike any tissue found either in *Fagus* or in *Epiphegus,* and is found only in the beech-root at the point of union. The growth of this haustorial organ always occurs at the ends in *Epiphegus,* not at the end in *Fagus.* And the beech-root remains very small and fine (Plate XXIX), even when it is attached to a great swollen parasitic tuber of the adult *Epiphegus.* It certainly seems that this organ of connection arises from the beech and grows into the tissues of the parasite. It will be assumed that this is the case in describing the structure, as it is the easiest method for description.

Near the point of connection the tissues of the beech-root assume a thoroughly disorganized appearance. The cells are not crowded together, but are rather loosened out, displaced and altered in aspect. There appear the short reticulated and spiral tracheids that characterize the haustorial tissue. Lying among these are the peculiar large, dense nucleated cells also found in the haustorial tissue. They are extremely large cells. Their nucleus is remarkably large and dense, and contains a small nucleolus. These two kinds of cells, the nucleated cells and tracheids, constitute the haustorial tissue, that shows exactly the same structure later, in its extreme ramifications in *Epiphegus,* as it does here. It is a unique tissue, there being nothing to resemble it in any structure of *Epiphegus.* In *Epiphegus,* the cells all show a definite arrangement. The tracheids lie alongside each other in long rows, and thus form continuous tubes. The nucleated cells also lie in regular rows. Probably the function of the tracheids is to pass over the flow of sap from the beech-root into the parasite. The appearance of the nucleated cells is certainly that of very actively metabolic cells. Quite probably they function in chemically transforming this beech sap so as to be used in the tissues of the parasite.

This peculiar tissue can be seen passing from the beech-root directly over into the parasite. At the point of crossing, a strong constriction is evident, the cells being greatly stretched out. The epidermis of the parasite and host is completely continuous along both sides of the connection. Immediately on entering, the haustorium divides, and sends two columns in exactly opposite directions. In tubers where the haustorium enters from below, these two arms go right and left in a horizontal direction. In the one being described, when the entrance was on the side, the two arms go above and below in a nearly vertical plane. The down-going column soon divides again, sending out two arms that diverge right and left in a nearly horizontal plane. After this there stream off various fine endings of the haustorium in various directions.

The up-going main column of the haustorium repeats the condition of the lower column. But it divides more irregularly and soon sends out long slender extensions that stream out and up in every direction. In all cases, the haustorial cells lie with their long axes parallel to the direction of each haustorial arm. The haustorium is always surrounded by several rows of simple flattened parenchyma cells. These may be *Epiphegus* cells compressed by the ingrowth of the haustorium, or they may be cells accompanying that organ. They are seen passing over into the beech-root. They are well marked in the region where the haustorium has just entered the parasite, and thin out to a single row in the fine haustorial ends.

These two haustorial columns formed in the upper region keep rising vertically for a considerable distance, after they have once reached the opposite sides of the tuber. Finally they are lost in fine endings. At this height the bundles have now assumed a rather regular arrangement, approximating that of the aerial stem. There is now a fairly complete ring of separate, undifferentiated bundles. These bundles are better developed on the side away from the haustorium. The haustorium finally has extended through fully two-thirds the entire height of the tuber.

At the summit and somewhat on one side of the tuber its growing apex appears. It consists of a considerable area of small, densely stained embryonic cells, containing large nuclei and nucleoli. Overlying this region, there are in such a young plant but four overlapping scale-leaves. These are relatively very large, and cover almost the entire width of the tuber.

The succeeding stages in the development of seedlings of *Epiphegus* have not been worked out. But from a brief examination of sections illustrating a number of these stages, it may be stated that on the lower part of the tuber new roots continually arise and elongate. The tuber elongates steadily, frequently bending its upper part around, if the young apex has been placed laterally, so as to give a true apo-geotropic growth. As it elongates, it keeps on differentiating in two distinct regions. The upper region develops numerous bracts, in whose axils flower-rudiments appear. In this region the bundles become steadily more regular and definite in arrangement. It finally bcomes the aerial stem of the mature plant.

The lower part, with its irregular bundles and emerging roots, becomes the tuber of the mature plant. The haustorial organ keeps growing in length mostly, pushing on through the tissues of the parasite and ramifying in all directions through the tuber. In the mature plant it is confined to the lower region of the tuber. The divisions and branchings of this organ keep increasing till finally there is produced the complicated, almost unintelligible structure of the mature haustorial connection of *Epiphegus.*

CONCLUSIONS.

1. *Epiphegus Virginiana* is a plant that illustrates in its various structures degeneration due to parasitic habits.

2. All evidence shows that it is parasitic only on roots of the beech-tree, and that it is annual in duration.

3. Seedling tubers appear in June, and steadily develop till August-October.

4. The vegetative part of the plant is the subterranean or semisubterranean tuber, the aerial portion and at times subterranean shoots from the tuber are reproductive.

5. Two distinct floral types—that are connected by transition forms—are observed, viz., the chasmogamic and the cleistogamic. Of these the cleistogamic is the commoner, and may alone occur on many plants.

6. Flowers of each type are confined to distinct areas of a plant. Chasmogamic flowers do not extend to the termination of branches, but beyond them are cleistogamic ones. (Gray, also Britten and Brown, leave it to be inferred that chasmogamic flowers are in the uppermost part.)

7. Chasmogamic flowers are equally numerous on plants growing in shade as in sunshine. A small percentage of them produces good capsules; not all are sterile, as indicated in botanical works.

8. The chasmogamic type of flower is the more primitive, the cleistogamic has been evolved from it by gradual modification of all its parts.

9. On the aerial parts stomata are abundant and widespread.

10. Bicollateral bundles are here frequent and well developed, while as in other parasites that have been described, the xylem is relatively small, the phloem relatively large in amount.

11. Complicated and anastomosing bicollateral vascular bundles occur likewise in the tuber.

12. The so-called "grapplers" arise endogenously, and are true roots, though by degeneration the root-cap has been lost. In structure they show degenerate histological peculiarities.

13. Histologically it is shown that the cleistogamic flowers are physiologically but not morphologically cleistogamic. They retain a fairly well-developed nectary that probably represents a fifth stamen.

14. The microspore follows the type of development common to angiosperms, but the mature grain shows division into two distinct nucleated protoplasmic masses.

15. The macrospore develops normally, but the endosperm nucleus produces a precocious endosperm, as in other related parasities, that grows up round the egg cell.

16. The developing embryo shows no trace of cotyledons.

17. The parasitic relation is established from the beech-root, rather than from *Epiphegus,* and is early shown as an invading ramifying tissue composed of large richly protoplasmis cells and tracheids, that eventually establish a highly complicated relation in the mature tuber of *Epiphegus.*

EXPLANATION OF PLATES.

PLATE XXIX.

Epiphegus Virginiana, parasitically attached to root of American Beech. Chasmogamic and cleistogamic flowers are both developed on this specimen.

PLATE XXX.

Fig. 1. T. S. of tuber, showing endogenously developing root. ×350°.

Fig. 2. T. S. of Beech root spreading into the looser tissues of the parasite. × 350°.

Fig. 3. L. S. of chasmogamic flower of *Epiphegus*, showing calyx, corolla, one of the four stamens and pistil. × 75°.

Fig. 4. L. S. of cleistogamic flower. × 75°.

Fig. 5. T. S. of ovary from cleistogamic flower, with large nectary, composed of richly protoplasmic cells, cut in section. × 350°.

Fig. 6. Young ovule or macrosorus, enclosing a large developing macrospore cell. × 350°.

PLATE XXXI.

Fig. 7. L. S. of developing macrospore cell, surrounded by [tapetal (?) and] sporangial tissue. × 350°.

Fig. 8. L. S. of developing macrospore cell, later stage, showing constricted protoplasm of the macrospore cavity, with two nuclei at either end.

Fig. 9. L. S. of micropylar end of macrospore or embryo-sac cell, showing two spindle figures at right angles to each other. × 350°.

Fig. 10. Innermost layer of macrosporangial cells, surrounding synergids and egg, large vacuolated endosperm cell with two fusing nuclei, also three antipodal cells. × 350°.

Fig. 11. L. S. of young ovule or macrosorus. The two endosperm nuclei have here fused. × 200°.

Fig. 12. L. S. of upper part of macrospore cavity, filled with endosperm cells that enclose a divided egg-cell. × 350°.

Fig. 13. Later stage than last, showing surrounding endosperm cells, tip of a pollen tube, suspensor cell and embryo cell with two nuclei. × 350°.

Fig. 14. Second division proceeding in embryo-cell. × 350°.

Fig. 15. Four-celled stage of embryo. × 350°.

PLATE XXXII.

Fig. 16. L. S. of sixteen-celled stage of embryo and suspensor. Eight of the sixteen cells and the four suspensor cells are seen in section. × 350°.

Figs. 17a-k. Stages in development of the microspores. ×350°.

The Histology and Development of Cassytha Filiformis, L.

(*WITH PLATES XXXIII, XXIV.*)

BY HARRIET BOEWIG, B. S.

The order Lauraceae is composed of green shrubs or trees of independent growth and normal nutritive relations, excepting in the genus *Cassytha,* which is unique in the extremely reduced condition of its vegetative system and in its parasitic habit. The latter peculiarity has attracted the attention of several observers, who have carefully investigated the relation of the plant to its host. Comparatively little has been done to the histology, and apparently no effort made to trace the relation of the seed to the adult.

It was mainly to complete the gaps in the history of this interesting genus that the present paper was undertaken, under the guidance of Professor Macfarlane, to whom I take this opportunity of expressing my indebtedness for the kind assistance he has at all times rendered me. The material for study was collected by him during the University's expedition to Florida in December and January, 1900-1901; and from fruits collected on the spot, seedlings have grown successfully in the University greenhouses.

The genus *Cassytha* is worldwide in its distribution in the tropics and sub-tropics. In habit it much resembles the stronger of our native Dodders, clambering over bushes so as to form masses several yards across. The color in hot, sunny exposures, as with *Cuscuta Gronovii,* is pale tawny yellow, but in shaded places it is of a rich dark green hue. All intermediate gradations were seen. The same plant on sunny and shady sides shows these differences most markedly.

The plant may be said to consist only of stem and branches, the leaves being scale-like and tiny. It forms an abundant cordage around the stems and leaves of the host plant. Very frequently the shoots coil tightly about each other, forming ropes and mattings, and then frequently parasitizing on each other. The internodes are long, but the stems branch freely. Connection with the ground is lost. The tangled mass is dotted over with short, thick spikes of flowers, the spikes being half an inch to an inch long, and the greenish white, sessile flowers three to four mm. across.

An exceptional feature of *Cassytha* is its apparently indiscriminate selection of hosts. In the Palm Beach and Miami neighborhood a great variety of plants were penetrated by it and more or less injured. It attacks most commonly and extensively the water oak (*Quercus aquatica*) and the evergreen oak (*Q. virens*), both of which become at times completely and closely wreathed over to a height of six to ten feet, young plants being partially or entirely destroyed. The plant is most abundantly encountered over tracks of sandy soil where scrub vegetation is fairly abundant. It may be said to occur almost continuously over the southernmost portion—130 to 140 miles—of Florida. In some localities it is somewhat local and irregularly distributed, as in the neighborhood of Miami. In other localities, as round Palm Beach, Neptune and St. Lucia, the plant is extremely abundant, though so far as observed it does not seem as yet to have become a troublesome infesting parasite to cultivated plants. A partial list of its hosts is given:

Pteris aquilina.
Zamia integrifolia.
Pinus (several species).
Carex (near triangularis).
Smilax Walterii.
Blue Palmetto.
Two species of Quercus.
Ceratiola ericoides.
Litzia Caroliniana.
Polygonella.
Cassia sps.
Bumelia argentea.
Yellow Coriopsis.

At times it runs along the white sandy ground a distance of eight or ten feet, becoming of a tawny yellow color, till it again reaches some host plant.

The plant will be treated of as follows:

1. Fruit and seed structure.
2. Seed germination.
3. Seedling growth.
4. Relation of the seedling to the mature plant.
5. Histology of seedling plant.
6. Histology of mature plant.

1. Fruit and Seed Structure.—Of the spike of four to eight flowers the lower ones mature first, and there seem to be considerable differences between the members of a single spike in time of flowering, as the lower fruits are sometimes fairly well on to maturity, while the upper buds are not yet open.

The fruit is of a clear, watery white color, generally spheroidal, and six to seven mm. in diameter when fresh. It dries to a brownish black color, the succulent pulp of the pericarp shrinking to a leathery consistence and somewhat reducing its size. The remains of the perianth segments are quite evident as little papery teeth at the "blossom end," directly opposite the point of attachment to the stem. Here the remains of the tubular calyx and the stigma sometimes also persist, the fleshy receptacle forming a pericarp or pseudocarp, which is attached to the fruit proper only at the base. The fruit is sessile, like the flower.

Fig. 1 (Plate xxxiii) represents the fruit in longitudinal section, and Fig. 2 a section of its walls in detail.

The receptacular tube consists of a rather tough epidermis of a single layer of cells without intercellular spaces, but with a few stomata (Figs. 1 and 2, 1); beneath which lie several irregular layers of loose, rounded cells rich in granular material (2), giving succulence to the fruit. Toward the interior these cells flatten somewhat and give place to large, irregular spaces (3), which in the younger stages show contents that take up stain, strongly suggesting mucilage. This layer is in every respect like the loose parenchyma of the leaf, and the one external to it like the palisade layer of the leaf. The endodermis is a sharply marked row of empty, brick-shaped cells.

The receptacular tube is fused with the fruit proper only at the lower end by an irregular growth of small cells from the one into the other.

The fruit and seed have been thus described by Bentham:*

"Fruit drupaceous, completely enclosed in the enlarged persistent and succulent perianth tube, usually crowned by the small persistent segments. Seed with a membranous testa. Embryo with thick fleshy cotyledons, distinct at an early stage, but completely consolidated when ripe, assuming the appearance of a fleshy albumen, at the base of which the plumula simulates the embryo."

The fruit shows an external layer or epicarp of brick-shaped cells, each with a faint nucleus and protoplasm (5). Within this are five to seven layers of thin-walled cells, the mesocarp, containing starch grains and chloroplasts (6). Internal to the last is a layer of shallow, greatly indurated cells, which in exact morphology should be regarded as the innermost of the mesocarp layers (7). It consists of a continuous line of square cells, clear-looking, with large round nuclei. As the fruit ripens, these remain unchanged below, and are distinguished from the more external parts by their regular and small size. Above the thickest part of the inner layer they rather suddenly elongate, narrow out and become filled with cellulose, the nuclei becoming obscure, until a cap forms over the top of the seed.

The remaining layer (8), from an early stage, is somewhat broader than the preceding. In the young state its cells are cubical and thin-walled in the basal portion of the fruit, with large round nuclei. Toward the upper end they become narrower, deep-yellowish, with thickening, and show elongated nuclei. With maturity this elongation and thickening takes place to a considerable extent throughout, but continues very rapidly at the upper end, so that in the ripe fruit a great cap of dense cellulose covers the upper end, four times the thickness of the cap produced by the inner layer of mesocarp. The cells have now become exceedingly narrow, with faint, disintegrating nuclei in the middle, the rest of the cell being filled with cellulose. While the inner mesocarp cells are usually quite brown in color, the endocarp is lemon yellow. These two layers, particularly the inner, invest the soft parts beneath with an armor of cellulose, which must be an excellent protection from all ordinary mechanical dangers. The only interruption to this is the minute aperture of the micropyle, which is filled with cellular tissue that will be referred to again. This aperture at the upper end has the radicle of the embryo extending vertically down from it and lying with the thicker or radicular end toward it, as shown in Fig. 2.

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* *Flora Australiensis,* Vol. V.

Internal to the horny cap is the outer indusial layer. It shows rather large columnar cells, very delicate-walled and empty (9). It fades out toward the micropyle. Within it, in a lateral position and almost or quite absent at the two poles, are several layers of loose, thin-walled empty cells (10), the tegmen. By the growth of the embryo these are crowded and partially absorbed until they leave only an irregular thin lamellated layer pressed close to the testa.

Next to the embryo, and separated from it by a mere basement membrane, is a series of obliquely placed spiral cells (11). These seem to form a very copious raphe and completely enclose the embryo. In the ripe seed they become narrowed toward the base. At the micropylar end they give way to a strand of fibrous tissue, which completely plugs up the end of the micropyle left by the fruit and seed coats. This strand extends down one side through the middle of the cellular tissue of the sorus. It is directly continuous with the style of the flower.

The embryo in the ripe seed is a globular yellow body consisting of two cotyledons that are not always of equal size, and that twist about each other somewhat; between them is a little doubly conical fleshy radicle, with papery indications of one or two plumular leaves. Bentham speaks on this subject as follows:*

"The cotyledons are so completely consolidated in the ripe seed that Gaertner described them as a fleshy albumen, mistaking the plumula, which is at least as much developed as in other Laurineae, for the embryo. R. Brown pointed out this error, and Griffith and others figured the real embryo with a distinct line of separation between the two cotyledons. In the dried fruits I had at my disposal, I could not detect any such demarcation, and I should have followed Gaertner in describing the seeds as albuminous had it not been for Brown's very decided contradiction, more especially as Colonel Champion, in some sketches made from the living plant in Hong Kong, seemed to confirm Gaertner's view. On writing, however, to Dr. Thwaites in Ceylon, he has kindly examined fresh seeds, and fully corroborates Brown's and Griffith's statements, explaining the discrepancies by the circumstance that it is only at an early stage that the cotyledons are clearly distinct, the line of demarcation becoming obliterated long before maturity."

To this I may add that the Florida material showed even in the fully ripened state a distinct line of demarcation between the two cotyledons, although they cannot be pulled apart as in the younger seed. I find further that the cotyledons remain distinct up to about the period when induration of the endocarp commences. I find, moreover, that the two cotyledonary epidermal layers facing each other remain quite distinct morphologically, but that they seem to be adherent by means of some cementing substance. These epidermal layers consist of fairly regular semi-columnar cells, whose flat faces are apposed, with the cementing substance between.

The bulk of the cotyledons is composed of large rounded cells, with scattered intercellular spaces. Each cotyledon is directly inserted into the radicle, which is entirely composed at this time of undifferentiated tissue. At the point of insertion some of the cells are a little elongated, and spread in four or five bundle-like traces from the radicle into the cotyledons, as is shown in Fig. 3. The two cotyledons are not, however, exactly opposite in insertion, hence the appearance of distinctness shown in the figure.

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* *Flora Australiensis,* Vol. V.

Both the cotyledons and the radicle are richly laden with ellipsoidal starch grains, protein material and less abundant globules of yellow oil.

Throughout the entire plant the starch is remarkably abundant and difficultly soluble. Continued boiling of thin sections with taka-diastase is the only means by which it can be satisfactorily removed. This is in all probability due to the superabundance even in the cotyledons of a thick mucilaginous material which cannot be localized in special cells, but which seems to be everywhere present. It swells and becomes very troublesome in weak alcohol, but is not dissolved by it, nor by soaking and boiling in water or KOH. Alcoholic material after a few months becomes somewhat granular, as if from coagulation of the mucilage, but when manipulated in weaker alcohol it swells and is as evident as before. It is not so stringy in fresh specimens as in alcoholic material.

There is no albumen; some starch is present in the middle layer of the receptacular tube, but this is a natural accompaniment of the chlorophyll present here, and cannot reach the embryo, owing to its isolation.

2. Seed Germination.—Hitherto the germination and seedling growth of this plant have not been studied. The germination of seeds was most successful in the two stove-houses of the Botanic Garden, at a temperature ranging from 88° to 90° F. The humidity was rather high, but it was found advisable to keep the soil fairly dry, as a constantly damp soil caused the formation of a white fungoid bloom over the surface of the fruit, and a blackening of the cotyledons with considerable or entire loss of roots and root hairs. These agree with the natural conditions of the plant. The best soil was almost pure sand. Under such conditions seeds germinated in three to four weeks.

3. Seedling Growth.—The radicle and hypocotyl find their way out of the seed by the micropyle, forcing the shell apart slightly by a single median split, which is, however, not at all extensive and exercises some elasticity, for while the hypocotyl when it first emerges is rather fleshy, it is not nearly so large as it becomes later on when entirely outside the seed. The lowermost part of the hypocotyl is a fleshy, cylindrical body, fifteen mm. long or less, of a pale yellow color, and is glabrous like the rest of the plant. As it emerges from the seed the food substance is rapidly passed from the cotyledons into it, causing it to become very turgid. The food is not reconverted into starch in the hypocotyl, but remains dissolved in the cell sap as sugar. At the lower end is a tiny root that is reduced to a mere conical tooth. In a circular zone above and around this, four side roots typically develop (Plate xxxiii, Fig. 4), which soon outstrip the main root and may reach considerable length. In some seedlings three, two (or even one) side roots develop, which are then somewhat stouter than when there are four.

Above, the hypocotyl attenuates rapidly into a thin, almost filamentous, but bright green stem, which is at first bent downward with its plumule in the seed (Fig. 4, a); it usually rights itself by the time its length equals that of the fleshy portion, and in most cases carries up with it the now empty seed (Fig. 4, b), which, because of its firm, elastic shell, is difficult to strip off. One seedling, fully six inches high, still bore the empty seed at its tip with the shell unbroken, and owing to the rather unfortunate growth of two leaves on the portion within the seed, these had become caught and the stem confined within had curled round one and one-half times inside the empty seed. Another seedling, on the other hand, appeared above ground without any seed coat (Fig. 4, c).

From the fact that the seed coat borne on the top of the seedling is always quite empty, and from the size and contents of the hypocotyl, as well as the embryonic appearance of the seedling, it seems highly probable that in the beginning of germination, and as soon as the hypocotyl is fairly out of the seed, all the cotyledonary food is passed into this lower portion previous to its being actually needed, and then the cotyledonary connection is broken. The scar of this separation is carried far up the stem by rapid and extensive elongation of the thin green portion. One seedling extracted itself from the seed when small and then continued growing, but the shell hung to the side of the seedling. Here connection had not been broken, although the seed was empty. The cotyledons were present as two papery flakes.

4. *Relation of the Seedling to the Mature Plant.*—The plumule usually bears two leaves of the characteristic small size. Owing to the great attenuation of both the hypocotyl and epicotyl, there is considerable space between the ground and the first leaf. In the case of the seedling that had been caught as above described, the distance was six inches. Haustoria may, however, form below the first leaves, as the bundle system is fully formed.

The seedling, as soon as it begins to grow erect, shows active circumnutating movements in clockwise direction. The sweep comes from a considerable distance down the stem and is fairly rapid. The seedling that was described as having been held within the seed wall and grown there to a length of nearly an inch, when freed, uncoiled completely and was beginning to circumnutate after ten minutes.

5. *Histology of Seedling Plant.*—The hypocotyl has a delicate epidermis with stomata, but very little cuticle. The fundamental tissue consists of loose, empty-looking cells, which are, however, quite turgid with sap and very rich in sugar. There are four patches of protoxylem evenly dis-

tributed, each consisting of from five to ten angular cells with lignified walls. External to each protoxylem patch and sometimes adjacent to it, or at other times separated by what seems a fundamental tissue cell, is a patch of delicate, thin-walled protoplasmic cells which feebly show wall markings in a few cells.

Between each two of these bundles, about evenly distributed, are two bundles of phloem which have no corresponding wood. These twelve phloem and four xylem patches are quite constant. Figs. 5, 6 and 7 show the distribution of these into the roots. The wood patches break up into smaller groups, and there seems a possibility of the elements anastomosing before they run into the smaller roots. The wood elements show chiefly spiral markings.

The roots have no root cap. They bear copious hairs, which are very turgid-looking, but quite short and mostly club-shaped. These extend to the very tip of the root. Sometimes the roots are ragged and broken off at the tip for want of a root cap. They attain no great length, a seedling nine inches long (Fig. 4, d) having roots half an inch long, somewhat tenuous, pale, almost transparent-looking, and very thin and feeble in appearance. The side roots are much more vigorous usually than the tap root. The seedling pictured had a larger tap root than is usual. In the tap root, in fact, growth ceases early; it usually remains a mere cone, and never has the appearance of a useful member.

*6. Histology of Mature Plant.**—*A mature stem* in cross section shows externally a fairly well cutinized epidermis of rather square cells, which have dense contents and contain nuclei (8, 1). Internal to this is a cortex (2) of four to six irregular layers, with intercellular spaces and which is dense with starch grains. The subepidermal layer is fairly regular. The cells also contain chloroplasts and considerable protoplasm with large round nuclei and nucleoli. Internal to this is a zone of hard bast (3), broken by rays of large cortical cells into smaller and larger patches, rather irregular in arrangement. The bast cells are somewhat larger than the phloem cells, but smaller than cortical cells and the lumen is reduced to a minimum. Beneath each patch of hard bast is an irregular space, formed by the degeneration of phloem, of which the flattened remains usually appear on the inner border of the space (Fig. 4), but which seem on the whole very feeble and unimportant. The rays separating these patches contrast somewhat with the outer cortical cells, with which they are continuous, in their larger size and comparative emptiness.

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*Since my studies on this were completed, a paper has appeared by A. T. Schmidt (in Oesterr Bot. Zeitschrift, V. 52, 1902), which treats of the structure of the mature stem.

The wood is arranged in a continuous ring beneath the phloem patches. In the old stem medullary rays are faintly recognizable. The wood elements grow larger toward the centre of the stem, and the most internal elements are very large pitted vessels, eight to ten in number (5).

Extending far into the pith are five to seven patches of delicate internal phloem cells (8), each patch enclosing three or four thickened cells of protoxylem (7), in rows or separate. The pith consists of large cells with intercellular spaces and without starch or protoplasmic contents. They have very thin walls and look quite empty.

A study of the parts of the mature stem in detail shows the epidermis to be provided with copious stomata, placed transversely on the stem, and arranged in longitudinal rows, which are slightly depressed below the surface, as has been already noted by Hackenberg * and other authors. As the stomata lie transversely, the cross section presents the appearance given by longitudinal sections of ordinary plants, and the usual appearance of two adjoining guard cells must be sought in the longitudinal section. The stomata are in

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* Verhandl. d. naturhist. Vereins d. Rhein. u. Westfalen, V. 6, Bonn, 1889.

other respects ordinary. The guard cells slope toward each other, have large nuclei and thick cuticle, especially toward the opening. The cells in the furrows connecting the stomata are somewhat concave in their upper surface, have thicker cuticle and are denser-looking.

The cortex shows, at irregular intervals in the subepidermal layer, large rounded isolated cells with dense yellowish contents concentrically arranged. Long-continued soaking and boiling in water failed to change the appearance of these contents. I have not been able to find that they extend over more than one cell in longitudinal section.

The hard bast forms perfectly homogeneous strands, each element of which appears uniform in longitudinal aspect. Sometimes their ends taper into each other. The bast was conspicuous for its impartial reaction to stain. Safranin and methyl green proved the best combination, as the red set off very sharply all wood elements against the green of all other parts. The hard bast alone stained red or green apparently without preference.

The outstanding feature of the wood in longitudinal section is the large pitted vessels. They appear in cross section as large round elements, frequently divided by branching into two irregular semicircles. In longitudinal section they are usually much broader than the adjoining elements, and the cells of which they are compounded show their ends broken through into a single large bordered pit with the membrane ruptured. The lateral walls show closely packed very large bordered pits, in various stages of growth. Fig. 9 represents a typical cell of the pitted vessels. With a one-twelfth immersion lens the middle lamella is seen to have quite measurable thickness, as is better shown in Figs. 10 and 11. It is easily enough seen, even with the low power lens.

Into these pitted vessels frequent and often very copious tyloses extend. These are most clearly seen when studied in longitudinal section in the two Figs. 10 and 11. The vessels are normally quite empty. Through their bordered pits from the adjoining smaller cells, hernioid protoplasmic swellings protrude, which even in the earliest stages show a delicate wall. This phenomenon is so frequent that one can scarcely cut a section without finding at least one instance of it. Not only the bordered pits, but apparently any of the pitted elements show it. The small undifferentiated wood cells are rich in protoplasm and contain healthy nuclei. They communicate with the large pitted vessels by the closely packed pits. One cell may have as many as six such openings, and through each of these the protoplasm may ooze. There is apparently no regularity, as the figures demonstrate. Frequently the entire nucleus, which in such cells always lies on the side by which the protoplasm is escaping, squeezes its way through the much smaller pit, and when it is through, again rounds out to its normal form. The entire vessel may thus be completely blocked by immigration of cells, which have nucleus and cell wall, though this latter seems of a highly plastic nature, being round when not impeded, but admitting of indentation by other buds that may come in contact with it. The small pitted cells into which tyloses form show no large connection between their ends, while the pits are oval, not bordered.

The protoxylem shows the usual spiral tracheae.

The leaves are small, scale-like and comparatively functionless. They are scattered and occur in a one-third spiral, six to eight cm. apart. The young green seedling has green, sessile scales, one to one and a half mm. long and with a broad attachment. They have stomata on both surfaces and are supplied with three veins. Some have long jointed hairs on their edges, probably surviving remnants rather than functional organs. The older leaves lose their green color, become quite membranous and dried at the tip, but retain their stomata.

In the seedling stem the earliest distinguishable trace of the stelar system is a ring of five xylem elements, each associated with a slight amount of phloem. Internal to these and widely separated from them by undifferentiated tissue are two solitary wood cells, irregularly placed. All these wood elements are decidedly thickened, although the primary membrane is well marked.

The protoxylem early splits up into three and much later into a greater number—six to eight—of patches. The protophloem patches remain five in number so long as there are three patches of protoxylem.

As soon as the phloem begins to develop hard bast, the true phloem disintegrates, and its place is taken by internal phloem. Thus are formed five canals external to the wood. These canals are more evident and considerably larger in the side shoots and tendrils than in the main axis or larger stems. In the larger stems there is also more hard bast than in the side shoots and tendrils. This seems to show that the reduction in amount of true phloem is not a mechanical result of the formation of bast, but is rather in line with the usual reduction of external phloem in twining stems. The internal phloem develops all around the protoxylem.

The wood spreads until its original patches are quite confluent around the stem, leaving no trace finally of recognizable medullary rays. By this time the external phloem, now consisting of little more than hard bast, whose cell lumen is also by this time almost completely obliterated, together with a trace of flattened phloem cells, has broken into perhaps twenty patches of varying size, large and small ones frequently alternating. The smaller of these have no canal and no soft bast. The internal phloem also consists of six to ten pointed triangular patches, each with two or three protoxylem cells enclosed.

The development of the bordered pits is clearly traceable through all stages and makes a very interesting study.

In young side shoots, and to a less extent in tendrils, the cortex remains more abundant and somewhat more columnar than in older ones.

Stomata are everywhere present and always in great abundance. On the leaves they are somewhat scattered, elsewhere they are in rows. They are also found on the petals, where they are in short rows. They likewise occur on the walls of the mature fruit, which represent the persistent receptacular tube. *Cassytha* is perhaps unique among the Angiosperms in having the stomata placed transversely to the long axis of the stem. The stomata themselves are rather small and of ordinary appearance. The origin of their transverse position, however, is interesting. On the hypocotyl, in the colorless and succulent region, they are sparse and are placed longitudinally. In the region where it attenuates and begins to look green, they are seen to lie obliquely and at varying and generally increasing angles, until in the green region they lie transversely. Quite young stem tips show stomata developing transversely. The epidermal cells, which in this region are nearly quadrangular in outline (Fig. 12, a), divide twice in succession, both divisions being perpendicular to the long axis of the stem. This forms four consecutive cells, which are considerably broader than they are long, and in cross section decidedly deeper than they are long. All four cells have sharply defined nuclei and a moderate amount of protoplasm. Fig. 12 b, shows this stage. The series of drawings in Fig. 12 is from longitudinal sections of the stem, therefore cross sections of the stomata. Safranin followed by methyl green causes the nuclei to become red or slightly purplish, the cell walls and cytoplasm green. It also stains the later thickening of the guard cell pink. Of the four cells thus formed the inner two are stomatic guard cells, and the further development of the stoma is a mere change of shape. The guard cells expand, squeezing the outer cells somewhat upward, so that they project slightly over the level of neighboring cells, and their nuclei are either much flattened, or they settle into the lowermost portion of the cell, the greatest pressure being upon its middle (Fig. 12, c).

Subsequent growth causes elongation of all epidermal cells, so that in older portions these sister cells no longer project above the surface. After spreading out, the stomatic cells flatten somewhat and lay down thickening on their outer face. This thickening assumes a slanting position (Fig. 12, d). The separation of the two stomatic guard cells, especially at their more internal portion, their further flattening and increased thickening of the upper wall, complete the formation of the stoma. Young stomata, even when completely formed, have no intercellular space beneath them. It is not at all unusual to find two or three stomata in a row, but they are always separated by two cells—the sister cells just described. Laterally consecutive cells do not form stomata. They are strictly in rows. The furrowing of these rows is not evident in young stems, and is a secondary development, apparently due to the greater expansion of parts not in line. This is further borne out by the hollowed appearance of the furrows. The cells do not sink abruptly, but are merely concave.

As will be seen from the highly organized stem system, this plant, while a typical parasite in many respects, cannot be said to have been much degraded in its stem histology. The extensive xylem system of the stem and branches, and its high organization of elements, as well as the abundant stomata and large amount of chlorophyll, indicate that the plant absorbs crude sap from its host and does much of its own elaborating. This position is further fortified by the structure of the haustoria, which show well-developed and abundant spiral tracheae running into their very ends, which curve directly into the wood area of the host.

That the chlorophyll is very active is shown by the superabundance of starch in the cells wherever chlorophyll exists.

The hard bast is a good source of support to a plant whose stems reach several meters in length, and which, owing to its trailing habit and mode of twisting into ropes, must often have considerable weight to support.

The large amount of mucilage produced is probably protective in character, as in the Cactaceae and other xerophytes. Cactaceous plants and *Cassytha* not unfrequently grow side by side.

The plant is not omnivorous in its parasitism, though the luxuriant appearance in its native haunts would lead one at first to suppose this. The seedlings raised in our greenhouses refused to attach to several hosts that were offered them, and seemed to prefer leguminous plants, though the long list of hosts observed shows that in the wild state it by no means confines its attention to this order.

Cassytha filiformis is thus a unique member of the Lauraceae and departs widely from its order in many structural details.

The writer hopes, in a later paper, to deal with the floral structure, which presents interesting peculiarities, particularly in its sporangial organs.

EXPLANATION OF PLATES.

Fig. 1. Longitudinal section of mature fruit.

Fig. 2. Portion of same, enlarged, somewhat diagrammatic. Reference figures apply to Figs. 1 and 2 alike.

1-4 Receptacle.
5 Epicarp.
6 and 7 Mesocarp.
8 Horny layer.
9 Indusium.
10 Tegmen.
11 Raphe.
12 Cotyledon.
13 Radicle.

Fig. 3. Cross section of embryo showing two cotyledons and connection of one by incipient bundle strands with hypocotyl.

Fig. 4. Young seedlings of *Cassytha*.

Figs. 5, 6, 7. Stages in the distribution of xylem and phloem strands into the four side rootlets.

5. Condition in the hypocotyl.
6. In the root, above branches.

7. In the root, at the point of origin of the side rootlets.

Fig. 8. T. S. stem.
1. Epidermis.
2. Cortex (chlorophylloid).
3. Hard bast.
4. Remains of protophloem.
5. Pitted vessel.
6. Tylosis.
7. Protoxylem.
8. Phloem.

Fig. 9. Pitted vessel, showing bordered pits in surface view and section.

Fig. 10. Pitted vessel and adjacent cells, showing tyloses.

Fig. 11. Same, showing extreme tyloses.

Fig. 12. Stages in development of stomata. Epidermis viewed in section.

Notes on Some Interesting British Columbian Plants.

By Edith M. Farr.

The plants on which the following observations are made were collected in the Canadian Rocky Mountains and Selkirks during the summers of 1903-04. The flora of these mountains is comparatively unknown and suggests many interesting questions. Although many of the plants seem at first sight to be like the eastern forms, a more careful examination often reveals marked differences, so that one is led to think that possibly many species west of the Continental Divide may vary from the typical eastern forms. At present there is no handbook which covers this special region, but I hope to continue my studies during another summer in the field to such an extent as to warrant the issuing of a Flora containing descriptions and illustrations of at least the more conspicuous plants to be seen at Banff, Lake Louise, Field, Emerald Lake, the Yoho Valley and Glacier, these being the principal places of resort along the line of the Canadian Pacific Railway.

I wish here to acknowledge my indebtedness to Prof. J. M. Macfarlane for the microscopical details mentioned in the accompanying notes.

Kruhsea Tilingiana, Regel,—3 inches to 6 inches high, slender, glabrous, simple. Roots filiform, three to four from each node of the filiform rhizome. Leaves, four to eight, ¾ of an inch to 2 inches long, ovate-lanceolate, acute at apex, the lower clasping at base, the upper semi-amplexicaul; one strong median vein, two feebler, lateral and additional, unequal veins; shining beneath, sometimes yellowish margined. Flowers about 4 lines in width, stellate in appearance, solitary in the axils of the leaves, borne on very slender decurved pedicels, the pedicel rarely provided with a lanceolate bracteole near the middle. Perianth tube very short, broadly campanulate, the segments ovate, acute, reflexed at the tip, the three outer (sepals) flat, the three inner (petals) falcate along the margins, wine-colored, the tips green. Stamens six, about one-third the length of the perianth, perigynous; anthers ovoid, bifid anteriorly, the walls strongly papillose over the upper half. Ovary provided with stomata, broadly ovate below, conical above, terminated by a minute, tri-lobate, stigmatic area. Style none. Berry 4 to 6 lines in diameter, at first obscurely three-angled, later globose, about twelve-seeded, bright red. Seeds oval to obovate, the outer, rounded surface ridged, the raphal surface flat, smooth, white.

Glacier; flowering specimens, June 30, 1904; fruiting specimens, August 22, 1904.

The present species and genus were defined by Regel in the "Nouv. Mém. Soc. Nat. Mosc.," XI (1859), page 122. The description is reproduced in Mr. J. G. Baker's monograph on the "Asparagaceae," published in the "Journal of the Linnaean Society," Volume XIV (1875) page 593, in which he implies that the plant was gathered in the flowering state, as he says, *"Baccam non vidi"*; but he seems to have overlooked the following sentence in Regel's description: *"Bacca rubra. Semina striata, albo-lutescentia."* On page 592 he describes *Steptopus? brevipes,* Baker, which was wanting in flowers, but which had fruit described as *"Bacca* 3—4 *lin. crassa, seminibus pluribus oblongo-clavatis."* Regel's and Baker's forms are evidently the same. My specimens were gathered around Glacier, B. C., in the Selkirk Mountains, at an altitude of 4,093 feet. Baker's *S.? brevipes* is recorded from "Oregon ad Cascade Mountains, 49° N. lat., Dr. Lyall." The distribution of *Kruhsea Tilingiana* is given by Baker as "Sitka, Eschscholtz; Ajan, Tiling; In ditione fluminis Amur, Maximowicz."

In the Gray Herbarium at Cambridge are specimens labeled *Streptopus brevipes,* Baker, which accurately agree with my *fruiting* specimens and with Baker's description of *S.? brevipes.* As pointed out to me by Dr. Rydberg, Curator of the Bronx Herbarium, my *flowering* specimens accurately agree with the elaborate description of Regel. The position, therefore, seems to be that a fruiting plant has been named *Streptopus? brevipes,* and the flowering condition of the same plant has been called *Kruhsea Tilingiana.* Apart from the priority of the latter name, sufficient distinctive features exist in the present plant to give it generic rank. The slightly amplexicaul leaves, the rotate, claret and green flowers with their perigynous stamens, the absence of a style and the very rudimentary indication of a three-lobed stigma, all contrast with the amplexicaul leaves of *Streptopus,* its funnel-shaped, white or pink flowers, the hypogynous stamens and the style with three-lobed or divided stigma.

In the "Proceedings of the American Academy" for 1879, Volume XIV, page 269, Dr. Sereno Watson states that Eschscholtz's specimens collected at Sitka and referred to *Kruhsea Tilingiana,* belong to *Streptopus roseus,* Michx. He continues, "On the other hand, the *Streptopus roseus* of Wright's collection in Ochotsk Sea is the same as Tiling's plant (from the same locality), upon which *Kruhsea* was founded, but is properly a *Streptopus* (*i. e., Streptopus ajanensis,* Tiling)." As Dr. Watson does not offer any evidence to establish his statement, the present specimens seem to confirm the identity of the series. From a phytogeographical standpoint the present specimen acquires exceptional interest as the plant is now recorded from Glacier (Farr), Cascades (Lyall), Sitka (Esch.), Ajan (Tiling), Amur (Maxim.). Probably owing to lack of fresh material, Regel, Tiling and Maximowicz have overlooked the strongly recurved, green-tipped condition of the perianth segments, their evident differentiation into sepals and petals, and the sessile stigma.

Lychnis attenuata, sp. nov.—Alpine, tufted, from a stout perennial root, about 6 inches high; branches diffuse, attenuate. Leaves 1 inch to 3 inches long, linear, mostly long-petioled, a few sessile, light green. Flowers about ½ inch long, solitary, nodding, borne on long, slender, pubescent, green peduncles. Calyx cylindric-campanulate, slightly narrowed at the mouth, copiously ciliate-pubescent, traversed by five strong and five fine longitudinal veins. Corolla slightly longer than the calyx, purplish-crimson. Petals elongate wedge-shaped, bifid and bearing small appendages. Stamens slightly exserted, five attached for one-third of their length to the petals, five free. Ovary about 4 lines long, ovoid, green with purple rim at the top. Styles about 3 lines long, exserted, the style tips yellow.

Lake Louise, near Laggan, July 16, 1904.

The nearest species to the above are the two already found and described as *L. apetala, L.* and *L. affinis,* Vahl. The present one differs in its attenuate habit, in the longer leaves, mostly provided with long petioles, the diffuse pubescence of the calyx, the deep attachment of five stamens to the petals, the exserted styles and the ovary which is intermediate between the two species.

In *L. affinis* the stamens are all free from the petals, in *L. apetala* five are slightly attached, in the present one five are attached through about one-third of their length; in *L. affinis* and *L. apetala* the calyx teeth are short, broad, in the present one they are broadly lanceolate.

It will thus be seen that the new form presents characters that in some respects are intermediate between the other two, and in others are entirely distinct.

Pachystima Myrsinites, Raf.—1 foot to 2½ feet high, branched, habit compact, internodes 2 to 4 lines long, twigs striate below, striate-verrucose above, dark brown or commonly black. Leaves 3 to 8 or rarely 10 lines long, coriaceous in texture, yellowish-green in color, from broadly oval to ovate, obovate and ovate-lanceolate, petioled, the petioles 1 to 1½ lines long, margins with five to nine short, blunt or slightly pointed teeth in the upper half, thickened and slightly reflexed; the midrib alone prominent on the upper surface, secondary veins indistinct, the lower surface reticulate; stipules minute, lanceolate, brown-black tipped. Flowers densely clustered in cymes; cymes three- to six-flowered, pedicels ½ to 1 line long, short, stout; bracteoles broadly ovate, truncate; flowers stellate. Sepals broadly oval, midrib faint. Petals rounded, nearly as broad as long, greenish-white tinged with purple. Stamens four, well developed, inserted into a prominent four-lobed disc. Ovary at first sunk in the hollowed out receptacle, later becoming prominent and green; style short, stigma capitate, papillose.

Cedar Creek, eastern slope of Selkirks, altitude 3,150 feet, June 15, 1904.

Pachystima macrophylla, sp. nov.—1 to 2½ feet high, branched, habit loosely spreading, internodes 5 to 10 lines long, twigs striate, cinnamon brown, traversed by brown-black ridges. Leaves 9 to 18 lines long, membranous-leathery in texture, bright green in color, ovate-lanceolate, rarely obovate, sessile or slightly petioled, margins five to ten-toothed in the upper half, sharply revolute; veins prominent on the upper surface, rather faint beneath; stipules minute, lanceolate, brown with black tips. Flowers arising as sparse axillary cymes in the axils of the leaves; cymes one- to three-flowered, pedicels 3 to 6 lines long, bracteoles ovate-acuminate. Sepals ovate with prominent midrib. Petals (as studied in material collected by Sandberg in Idaho) ovate to ovate-lanceolate, two to three times longer than broad. Stamens four. Pistil as in *Myrsinites.* Fruit 3 lines long, inequilateral through abortion of one carpel, dehiscence loculicidal along the lateral superior face. Capsule one-seeded, seed oval, slightly ridged, mahogany brown in color, surrounded by a membranous split up aril and suspended for a time by a long funiculus.

Bear Creek, eastern slope of Selkirks, altitude 3,670 feet, August 20, 1904.

As originally defined by Rafinesque and Pursh it is scarcely possible accurately to determine which of the above species is intended from the descriptions, since the salient points of distinction are entirely overlooked. In the more recent descriptions of Trelease and Howell, and also from the study of specimens distributed by Howell, the specific name *Myrsinites* should be restricted as above given. The flowering period of both is about the middle of June. Although not included in the definition there seems a strong reason for believing that the flowers are gyno-monoecious, there being a few blooms with large protruding ovary and rather rudimentary stamens, while the majority of the flowers have well-developed stamens and a deeply-sunk receptacular ovary. Future study alone can settle these points. From all the material collected by the writer and examined in herbaria, the flowers of each succeeding season seem to develop in the preceding autumn, and remain in bud condition throughout the winter, much as in Dogwood and Japanese Paulownia. A study and comparison of these forms over wide areas of Western North America is highly to be desired.

In order to determine more accurately possible points of difference between these species, a histological study has been made with the following results:

Pachystima Myrsinites, Raf.—Cork broad, black externally in a broad zone, brown internally in a rather narrower zone. Cortex with few conglomerate crystal cells, sclerenchyma strands in the collenchyma feeble or mostly absent. Sclerenchyma elements of inner cortex scattered in patches of eight to five or even solitary, but in a pretty continuous line. Wood dense. Pith cells all heavily indurated; abundantly starch-storing. Leaves with lower epidermal cells one-third smaller than next, walls straight or slightly wavy and stomata relatively abundant; the median vascular bundle of the midrib with a sclerenchyma strand beneath the phloem that is comparatively small in amount, leaf below the strand flat and with epidermal cells scarcely swollen.

Pachystima macrophylla, sp. nov.—Cork tissue brown or with a thin brownish-black external layer. Cortex abundantly provided with conglomerate crystal cells, strong strands of sclerenchyma in the colloid layer of the cortex and small strands in the inner cortex. Wood open, porous, with relatively large pitted vessels. Pith relatively small, the central pith cells moderately thickened with punctations; starch rare or absent in these. Leaves with lower epidermal cells of larger size and more strongly sinuous walls than in *Myrsinites,* the stomata one-third fewer, sclerenchyma strand two to three times larger, leaf below the midrib strongly swollen and the epidermal cells expanded into papillae.

Cornus Canadensis, L. var. *intermedia,* var. nov.—Stems 5 to 7 inches high, usually simple, rarely branched, woody at base, from a creeping, horizontal rhizome. Leaves either mostly verticillate, two to six at the summit of the stem, with two much smaller leaves and scale-like bracts below, or occasionally borne in pairs, nearly sessile, oval, ovate or obovate, acute at each end, or sometimes rounded at base, entire, glabrous or minutely appressed-pubescent, with two much smaller leaves and scale-like bracts below. Flowers purple, capitate, subtended by four involucral bracts, 4 to 9 lines long, ovate, white, petaloid, somewhat unequal; borne on slender peduncles 6 to 18 lines long. Petals white with purple tips, ovate, one of them with a subulate appendage at apex. Stamens alternate with petals. Styles much exserted, deep purple, giving a dark appearance to the flower. Fruit about 2 to 4 lines in diameter, globular, bright red, appressed-pubescent, one-seeded, seeds ridged on either side.

Glacier, flowering specimens, July 5 and August 12, 1904; fruiting specimens, August 22, 1904.

Specimens of the above *Cornus* were gathered during the past summer at Glacier, which alike from study of fresh material in the field and detailed laboratory examination has proved a puzzling form. It seems in combination of characters to unite to a remarkable degree the specific characters of

C. Canadensis and *C. Suecica.* Material hitherto collected in this region has been reported as *C. Canadensis.* Many of the flowering shoots bear fully developed opposite pairs of leaves, but without trace of whorled leaves. In shape and size these approximate rather more to those of *C. Canadensis,* but not infrequently their bases are rounded so as to approach very closely those of *C. Suecica.* In diagnostic descriptions the leaf veins of *C. Canadensis* are said to be pinnate and this is generally true; in *C. Suecica* the veins are said to arise at or near the base of the leaf, but leaves are not infrequent that show marked pinnation; in the present form the veins occasionally arise near the base of the leaf. The flowers of *C. Canadensis* are described as greenish, those of *C. Suecica* purple. The present specimens conform to the latter description, and this has already been noted by Pursh, who says in respect to *C. Canadensis* "flowers purplish-white." The stones of *C. Canadensis* are stated to be smooth, globose, a little longer than broad, those of *C. Suecica* are defined as flattened, channeled on each side and about as broad as long. The stones of the specimens now under consideration exhibit an intermediate condition, but approximate rather more to those of *C. Suecica.*

In view of these marked variations, it seems quite reasonable to consider that the Glacier plants represent perfect transitional types in every detail from the two supposed species, and verify the wisdom of the observation made in the "Botanical Magazine," No. 280, that *Cornus Canadensis* "is for the most part readily distinguished from *Cornus suecica,* by the leaves all growing in a whirl at the top of the stem, for the opposite pair about the middle are mere stipules; Pallas doubts if they are not both varieties, and says the specimens he has seen from Kamschatka and Bering's Island, exactly correspond with garden specimens from this country and native ones from Canada, but it is not very improbable that *Cornus Canadensis* may be found in these places as well as *Cornus suecica;* we have specimens of both from Labrador. The other distinction which Willdenow observes of the leaves in one being *nervosa,* in the other *venosa,* remarked also by Mr. Salisbury, in Smith's Flora Britannica, though not unfounded, is hardly sufficiently pointed for use, as the nerves in *suecica* sometimes take their origin from the midrib, and the veins in *Canadensis* are so strongly marked on the under side and so little divided, that most describers would call them nerves."

After due consideration of these various points, it seems advisable to make the present form a variety of *C. Canadensis,* as it resembles that species rather more than *C. Suecica.*

Corallorhiza innata, R. Br. var. *virescens,* var. nov.—This variety differs from the type in the color of the flowers, which are a light yellowish-green.

Banff, Alberta, June 14, 1904; Field, B. C., June 11, 1904.

Senecio triangularis, Hook.—The typical form of the above species is abundant at Glacier, Lake Louise, and over other areas of the region now dealt with, but along the lower part of the Asulkan Trail it occurs interspersed with a remarkably luxuriant variety that attains a height of 4 feet, which bears ample leaves that are three to five times the size of the typical form, but which most strikingly varies in the rudimentary condition or absence of the ray flowers in the capitula. The entire plant also is more hairy in character.

The History, Structure and Distribution of Sarracenia Catesbaei, Ell.

By J. M. Macfarlane, D. Sc.

Sarracenia Catesbaei was first described by Dr. Stephen Elliott in "A Sketch of the Botany of South Carolina and Georgia." In Volume II, page 11, he defines it as follows: "Leaves firmly erect, tube funnel-shaped, longitudinal wing linear, throat straight, appendix erect, somewhat reniform, reticulate with colored veins." He then refers to Table 69, Fig. B, of Catesby's "Illustrations," and adds: "Leaves 12 to 18 inches high, regularly tapering to the base; the upper part of the leaves and the appendix distinguished by their colored veins, the inner surface of the appendix covered by long and very conspicuous hair.

"This plant which has probably been united with the *S. flava*, and which can be connected with no other species, appears to me sufficiently distinct; it differs by its rigidly erect leaves, by its throat, which is straight and not expanding, and by its appendix, of which the sides are not reflected. It differs also from the *S. flava* by its darkly colored purple veins and heavy appendix. My specimens agree exactly with the figure in Catesby, to which I have referred, and were collected by Dr. Macbride along the margins of the rivulets amidst the high sand hills of Chesterfield district in South Carolina. The flowers I have not seen."

On reference to Catesby's original description—which he applies to *S. flava*—it is said "The leaves of this plant are tubulous and ribbed, arising from a knotty fibrous root to a height of about three feet; they are small at the root, widening gradually to the mouth of the tube, which, in young leaves, are closed, but open by degrees as the leaf increaseth; and when near its full growth arches over the mouth of the tube in the form of a friar's cowl. This cowl expands itself till the leaf is at full bigness, having its inside of a yellowish green, veined with purple."

Catesby's herbarium specimens are now deposited in the herbarium of the Botanical Department of South Kensington Museum, and an inspection of these during the past Spring proved that both accurately agreed with Catesby's drawings and belong to the species *S. flava.* Elliott was therefore mistaken in founding his species on any specimens collected by Catesby. I have been unable as yet to learn where Macbride's specimens, sent to Elliott, were deposited and if they are still in existence. But in his monograph of the genus, Croom says (page 104): "Elliott's Sarracenia, *Catesbaei,* is, as I have ascertained by the inspection of his herbarium, scarcely even a variety of this species, and differs from the ordinary form of the plant only by the more conspicuous veins and pubescence of the lamina. It agrees very well with the figure in Catesby, which Elliott refers to his *S. Catesbaei,* while both Willdenow and Pursh quote the same figure as belonging to *S. flava.*"

Founding, therefore, on Elliott's description alone, a species is indicated by "its throat which is straight and not expanded, by its appendix of which the sides are not reflected," and it is further distinguished by its "hairy appendix." Elliott is in error, however, when he says "it differs also from the *S. flava* by its darkly colored purple veins," for in this respect, varieties of *S. flava, S. Catesbaei* and *S. rubra* may perfectly agree. The next reference to the plant is made in Nuttall's paper of 1830 on *S. calceolata,* where he associated in one generic subgroup "*S. flava* and *S. Catesbyana,* lately restored by Mr. Elliott. In these the flowers are yellow."

In the seventh edition of Eaton's "Manual of Botany" (page 508), *S. Catesbaei* is defined as having "leaves stiffly erect, tube funnel-form, lateral wing linear, throat straight, appendage erect, subreniform, reticulate with colored veins." This description is evidently drawn from that of Elliott.

In the "Bulletin of the Torrey Botanical Club," Volume XXIV, 1897, are: "Notes on some undescribed and little known plants of the Alabama Flora," by Dr. Charles Mohr. On page 23 he refers thus to *Sarracenia flava Catesbaei* (Elliott): "Near the type differs in habit of growth and range of distribution. Leaves rarely over 12 inches long, with a very narrow wing, erect hood, dark purple veined; the lamina covered with a fine silky pubescence, apparently confined to the mountains of South Carolina and Alabama. Alabama, DeKalb County, Look-out Mountain, bank of Little River, about 1,700 feet."

In Heller's "Catalogue of North American Plants," Second Edition (1900), page 92, *Sarracenia Catesbaei,* Ell., is recorded.

In Mohr's "Plant Life of Alabama" (1901), page 131, *S. Catesbaei,* Ell., Catesby's Trumpet Leaf is given by him from the same locality as above. The addition, however, is made to the former description, "flowers yellow, June." On page 79, he says, *"S. Catesbaei* and *Isoetes Engelmanni valida* are paludial plants, so far only known in the state from the banks of Little River near DeSoto Falls."

Small in the "Flora of the Southeastern United States," page 484, describes it as follows: "Leaves erect, rather slender, narrowly trumpet-shaped, 2 to 5 decimeters long, slightly expanding above; hood ovate, with relatively straight inconspicuous veins, these much less branched than those of *S. flava;* scapes about as tall as the leaves; sepals widest near the base, tapering to the narrow but blunt apex, resembling the petals in texture; petals greenish yellow, 5 to 6 centimeter long, fiddle-shaped; blades rhombic ovate, the abruptly widened basal portion nearly 2 centimeter long, rhombic obovate; capsule similar to those of *C. flava,* but with longer processes. In swamps. South Carolina to Georgia and Alabama. Spring."

The above observations suggest the possible existence of a species with yellow flowers, distinct from *S. flava* and *S.*

variolaris. As already shown in part, and as will be shown hereafter, these descriptions are by no means satisfactory, and even in some cases incorrect.

Reference may now be made to the fact that during the last thirty years at least, to the writer's knowledge, specimens have been grown in various of the European botanic gardens, under such names as *S. flava picta, S. Fieldsii,* etc., which showed puzzling divergencies from the two well-known yellow-flowered species *S. flava* and *S. variolaris.* From fifteen to twenty years ago the writer's attention was attracted to several large pots of these that grew in the Sarracenia House of the Edinburgh Botanic Garden. The pitchers in general aspect, and in microscopic study, very closely resembled strong examples of the crimson flowered species *S. rubra.* The flowers were about three times larger and of a pale sulphur yellow. Fruitless efforts were made to ascertain their origin either as wild plants or as possible hybrids.

Matters so rested till nearly two years ago, when the writer received a rich supply of Sarracenias from Dr. Sledge, of Mobile, Ala. All were in the fruiting stage, but the general resemblance of the pitchers to specimens of *S. rubra* or even more to the Edinburgh specimens of *S. flava picta,* were immediately noted. Microscopic study and comparison of these with pitchers of *S. rubra,* showed the two to be almost identical, and very different in details from all of the other species. Their flowering period was therefore awaited with interest. Meanwhile through the kind and sympathetic assistance of Provost Harrison, the writer was enabled to visit the Mobile region, from which the specimens had been secured, and to obtain a fresh supply for the University Garden, which was later supplemented by a donation of specimens from Mr. H. G. Gayfer, of Mobile. The specimens collected by the writer were gathered on the 5th of February, of the present year, and these showed flower stalks about 2 inches long. They were placed in the Sarracenia House of the University Botanic Garden, and began to bloom within three weeks. They, as well as the Sledge and Gayfer specimens, continued to bloom throughout the succeeding month, each flower lasting, on the average, for seventeen days. They varied from a pale lemon-straw color to almost white, and in size were intermediate between those of *S. flava* and *S. variolaris*. Their identity with *S. flava picta* of the Kew, Edinburgh, Glasgow and other botanic gardens was fully established.

A review of the past descriptions of the species may now be given as follows: Elliott's short, but exact description of the pitchers, strongly indicates that his specimens represented a form distinct from, but which up to that time had not been distinguished from, *S. flava*. Nuttall * accepted Elliott's diagnostic description and further added that the flower is yellow. Eaton's description is equally exact, though it seems to have been drawn from that of Elliott or Nuttall. Mohr seems to refer to *S. Catesbaei*, but the specimens from Little River near DeSoto Falls are unquestionably those of *S. flava*, as the writer has had the opportunity of inspecting these through the kindness of Dr. Smith, head of the Alabama Geological Survey. Small's description, which is the fullest hitherto published, is thoroughly diagnostic, but statements made by some of the writers might suggest that they were dealing with natural hybrids, which are not uncommon in the Southern States. Thus at least one would very closely apply to wild hybrids between *S. flava* and *S. variolaris*, such as the writer has collected near Summerville, S. C. That similar hybrids have been already sent out under mistaken determination, is shown by sheets in the United States National Herbarium, the Gray Herbarium at Harvard and the Berlin Herbarium, distributed to two of these places by R. M. Harper as *S. Catesbaei*, but which are evidently natural hybrids of *S. Drummondii* and the crimson throated form of *S. flava* that is common in Georgia.

After a careful study of the species, alike in the field and under cultivation, the following diagnostic description can be given:

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*Trans-Amer. Phil. Soc. V. 4, p. 49.

Rhizome stout, elongated; leaves erect, 30 to 75 cm. high, gradually expanding into a pitcher upward, veins prominent, median flap widest in the middle, tapered below and above into a slight ridge; pitcher rim narrow, sharply recurved, orifice 2½ to 3 cm. across; lid ovate-cordate, nearly erect or arching, base of lid flat or very slightly recurved; inner surface traversed by radiating purple veins, and sharply divisible into an attractive surface over its upper two-thirds and conducting surface over its lower third, attractive surface covered with fine, evident down-directed hairs; base of lid and upper interior of tube with longitudinal purple veins and oblique, intermediate, reticulate veins. Flowers nearly equal to or slightly longer than the spring leaves, decurved, from lemon-yellow to nearly white, 5 to 6 cm. broad. Sepals ovate, narrowed at base, 3.5 to 4.5 cm. long by 2.5 to 3.5 cm. broad, at first greenish-yellow, becoming lemon or pale yellow during flowering; petals fiddle-shaped with strongly recurved edges, at the median constricted part 4.5 to 7.5 cm. long by 2 to 3.5 cm. at widest portion, rounded at the free end. Style pentagonal-repand, 4 to 7 cm. across; style arms beyond stigmas 4 mm. Capsule 1 to 1.5 cm. across. Flowers lasting about seventeen days. Odor delicate, agreeable.

The annual duration of the leaves of this species stand in the following relation to the other six species of the genus. Those of *S. flava* spring up in April, and are largely withered and brown in October; those of *S. rubra* last till the end of November or into December, the autumn leaves of *S. Drummondii* last till February or March of the succeeding year; those of *S. Catesbaei* remain green and fresh till the end of April, when a new crop has been produced; those of *S. variolaris, psittacina* and *purpurea* last still longer and in the order indicated.

Nothing has been learned as to the source or time of distribution of the living plants that are found in the European botanical gardens. The writer has been informed, however, that about thirty years ago, when the plants enjoyed great popularity through the observations of Mellichamp and Hooker, considerable supplies of the different species were gathered round Mobile and sent to European gardens. Now *S. Catesbaei* is abundant over some parts of this area, and may have been included in the shipment.

The species is well represented in American and European herbaria. Thus in the United States National Herbarium are nine sheets collected from widely different localities. In the Gray Herbarium at Harvard are four sheets, in part duplicates of the last, in part from other localities; while in Pennsylvania University Herbarium are two from distinct localities. In Kew Herbarium are four sheets chiefly from regions round the Gulf. In South Kensington Herbarium are three sheets, while representatives are also to be found in the Florence, Leyden and Utrecht herbaria. In nearly all of the above the sheets were labeled *S. flava*.

Physiologically the present species is of some interest, as it is the most successful fly-catching member of the genus, alike under cultivation in greenhouses and in the open. This is due in part to the abundance of the nectar secretion, and in part to its long continued secretion throughout the season. Mellichamp rightly observed of *S. variolaris* that its attractive secretion lasts for a comparatively short period during spring, that of *S. Catesbaei* continues often for months. Though the drops exuded are not so large as those over the outer surface and lid margin of *S. flava,* the concentrated quality of the secretion and its long continuance seem both to be pronounced.

S. Catesbaei seems to be distributed over a wider area than are several of the Southern species. It was secured by Dr. Sledge in abundance about six miles below Mobile along various "branches." In company with Mr. H. G. Gayfer I had the pleasure of observing and collecting it along the northeast side of branches of the Dog River below Mobile, and it may possibly be from this locality that it was recorded by Jewett in 1838, and by Sullivant in May, 1845. The earliest specimens of which I have exact knowledge were collected by T. Drummond at New Orleans in 1832, and these constitute his No. 12, sent out under the name of *S. flava,* Mich. It is recorded from near Mississippi City, Miss., by W. M. Canby; from Biloxi and from Ocean Springs, Miss., by S. M. Tracy; from Hammond, La., it has been sent out by L. Gallup; from Texas specimens have been collected at Swan by Reverchon, and at Kountze by G. C. Nealley. A sheet of specimens, No. 284,926, of the United States National Herbarium has been collected by Prof. L. F. Ward near Florence, S. C. This locality is the nearest at present known to that from which Macbride secured his specimens "along the margins of the rivulets amidst the high sand hills in South Carolina." From information received from various correspondents it is specially abundant from Alabama westward to Eastern Texas, and is particularly so along the costal belt. Records seem to be wanting for it over Florida, Georgia and the greater part of South Carolina.

The resemblance of rather small sized pitchers to those of *S. rubra* is striking, and this extends also in a minute degree to the microscopic structure which I hope to treat of elsewhere, while both differ in this respect from the other species of the genus. The flowers are highly diagnostic, for in the soft petaloid character of the sepals, and their resemblance in color to the pale lemon-yellow petals, they markedly differ from those of *S. flava.*

As regards flowering periods, *S. Catesbaei* is the earliest species. The writer gathered material near Mobile in February which already showed flower buds about two inches in height. From records kindly supplied by Mr. H. G. Gayfer these blossomed in the second week of March, while flowered herbarium specimens from various other localities along the coast record dates from March 19th to April 3d. The specimens collected by L. F. Ward, near Florence, were in bloom on April 17th. The next species to flower is *S. flava,* which is a week to ten days later at corresponding localities; succeeding it is *S. variolaris,* still later *S. psittacina* and *purpurea,* while *S. rubra* and *S. Drummondii* bloom from three weeks to a month later than does *S. Catesbaei.*

Though other diagnostic points were wanting, the odor of blooms of *S. Catesbaei* and *S. flava* suggest decided differences. As has been observed by Torrey those of the latter are heavy, disagreeable and suggestive of a compound of Catnip and Turkey Rhubarb; this, moreover, is specially strong in an April evening from 7 till 10. The odor from the former suggests a delicate lemon-violet combination. It would be instructive to know what insects frequent these blooms habitually in different localities, for while the writer has gathered some statistics, they are fragmentary and should be widely supplemented.

Special acknowledgments for generous help are due to Dr. Sledge and his friend Mr. H. G. Gayfer, of Mobile, who have in every way exerted themselves to secure material and information, and who kindly introduced the writer to native localities for the species. I desire also to thank the Directors and Curators of the Botanic Gardens and Herbaria above mentioned for courtesies extended.

Statement Regarding Publications from the University Botanical Department.

By Prof. J. M. Macfarlane.

The contributions from the Botanical Laboratory of the University have hitherto been mainly devoted to publication of morphological and physiological papers by various of the workers. With the steady growth of the Botanical Garden, the Herbarium and the Library, facilities have been secured for successful prosecution of all lines of botanical study. In order to utilize these to the utmost the Professor of Botany has arranged for the preparation of a series of monographs dealing with special plant groups. These will be published either in the "Contributions from the Botanical Laboratory" or by other recognized means of publication, and will permit the continuation of the valuable exchanges that have been already established.

Each monograph will deal fully with the history, morphology, physiology, taxonomy and ecological distribution of the species reviewed, and will be copiously illustrated by black and white, also by colored drawings. The subjoined are now in preparation:

(1) "Water Lilies—A Monograph of the Genus *Nymphaea.*" By Henry S. Conard, Ph. D., late Harrison Fellow in Botany.

(2) "A Monograph of the Order Ascidiaceae, including the Sarracenieae and Nepentheae." By J. M. Macfarlane, D. Sc., Professor of Botany in the University.

(3) "The Genus *Pentstemon*—A Monograph of the Species." By Louis Krautter, B. S., Assistant in Botany.

The first of these will shortly be published by the Carnegie Institution, and has been prepared during the past five years under the following conditions: Henry S. Conard, M. A., was appointed Harrison Fellow in Botany in 1899. The above subject was outlined and assigned to him, along the lines as now being published, by the Professor of Botany, the entire study being intended as an original investigation and thesis. From the abundant facilities furnished by the University Garden and the Dreer collection of water lilies, the subject grew in importance, so that the author felt warranted in presenting the taxonomic part alone as a thesis for the Ph. D. degree. He was then awarded the Harrison Senior Fellowship in Botany for two years, and owing to the ample leisure that this afforded he was enabled to complete the original study in its entirety. This was presented to the Carnegie Institution by the writer and was duly accepted for publication. Through the interest of Dr. S. Weir Mitchell, and on permission from Provost Harrison, Dr. Conard then spent some time in visiting the European herbaria on the Carnegie foundation. He thus secured additional information, which has been incorporated in the work that will shortly appear.

The second of the above monographs is in preparation, and will embody the results of study in the field, the laboratory, the herbarium and the library of the past twenty years. Through action of the Provost and Trustees of the University the writer was able to devote considerable time during the past spring and summer to investigation of the group of the Ascidiaceae in European institutions. The work will incorporate these results, and will, it is expected, be published as a University Contribution.

Mr. Louis Krautter's investigation is now in progress and will include a historical, morphological, physiological, taxonomic and ecological study of the above suggestive genus of North American plants.

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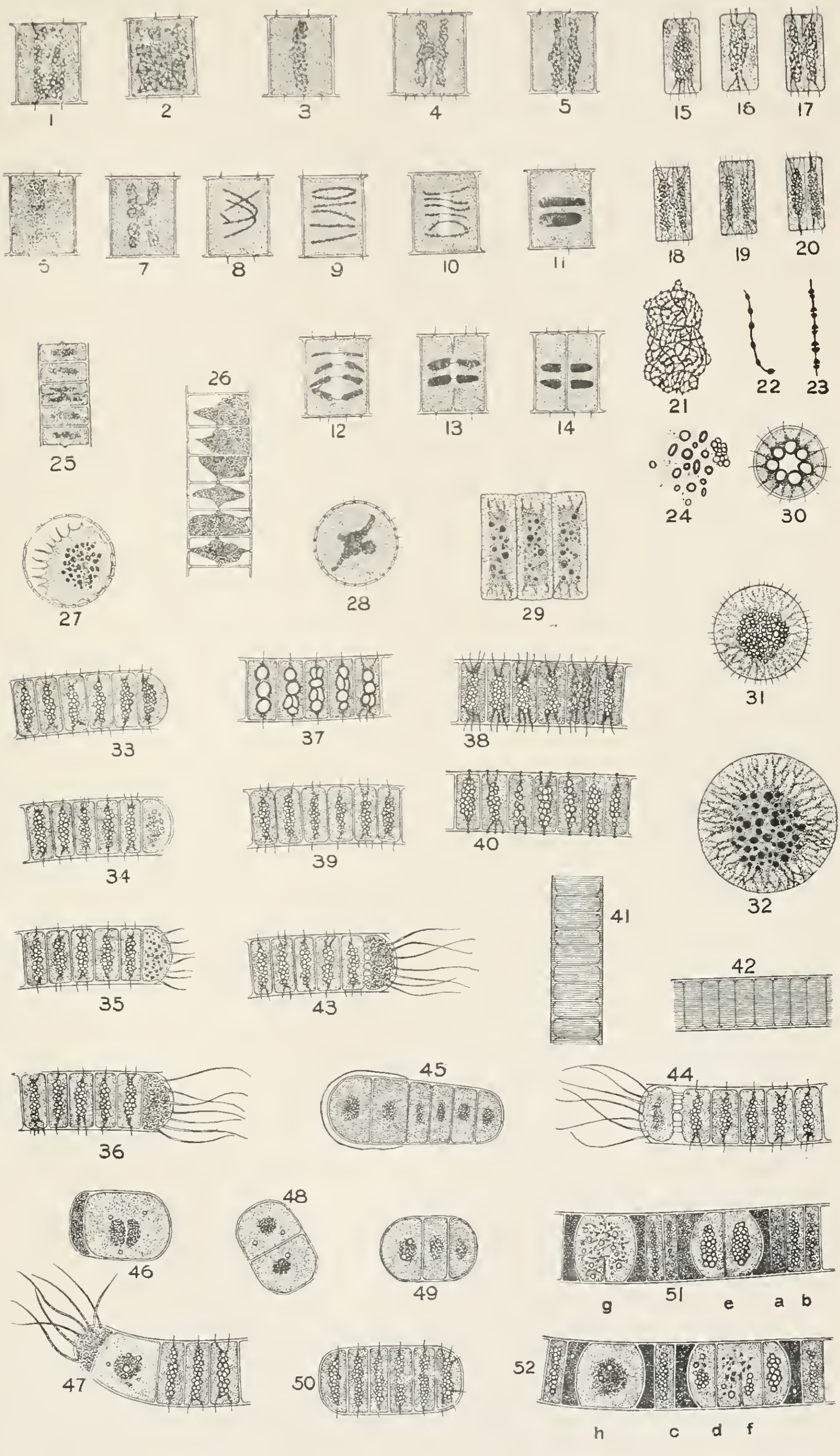
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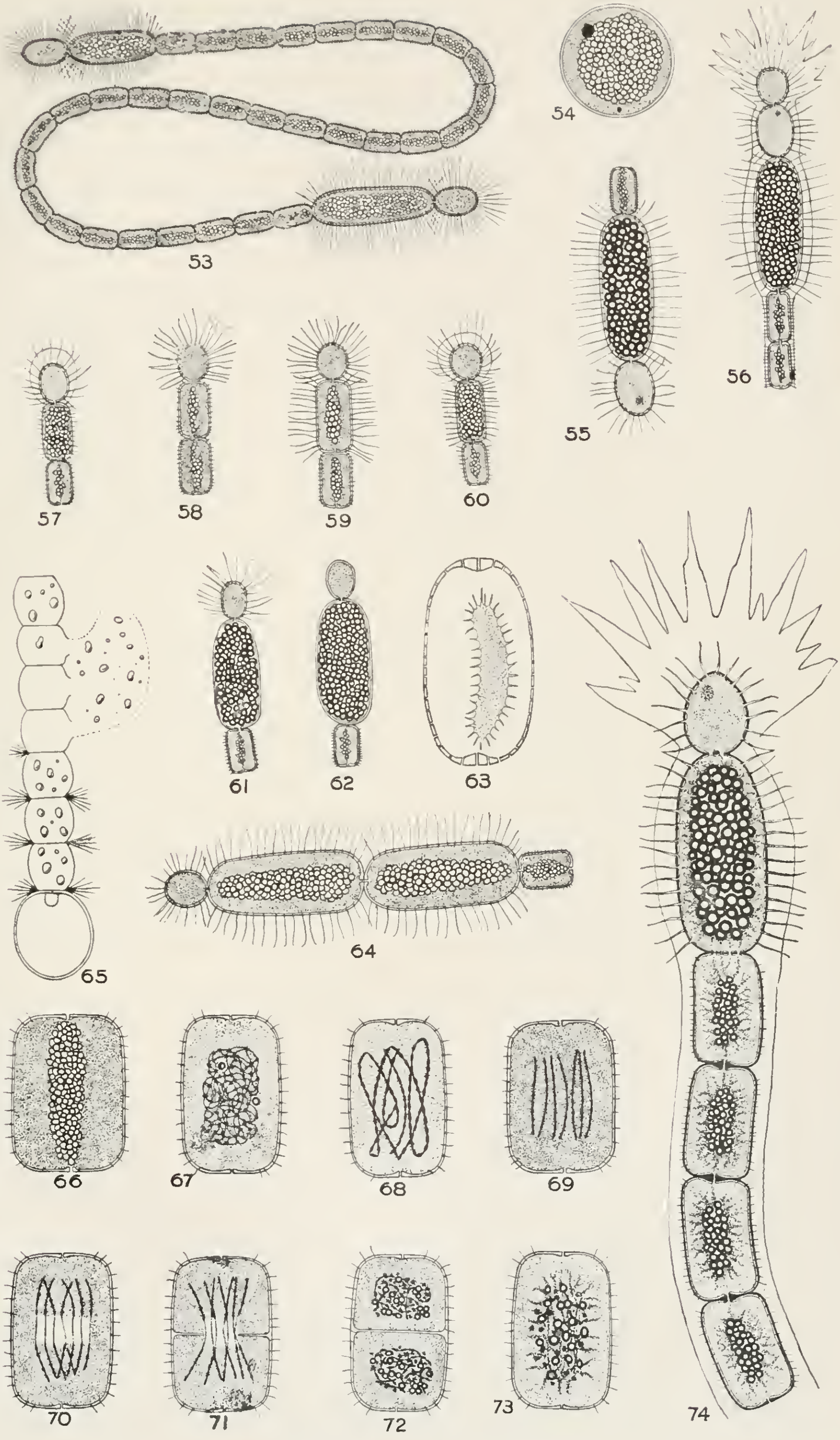
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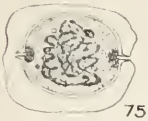
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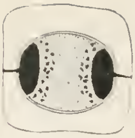
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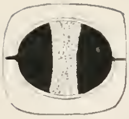
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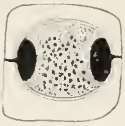
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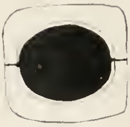
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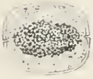
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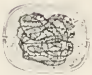
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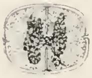
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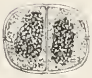
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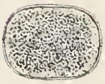
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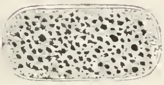
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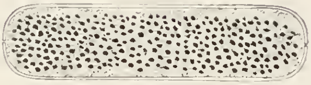
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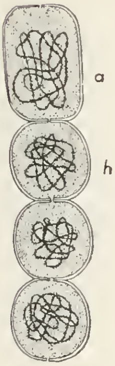

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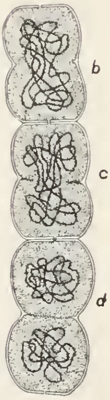

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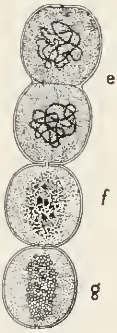

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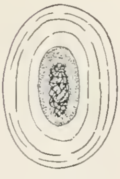
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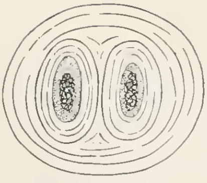
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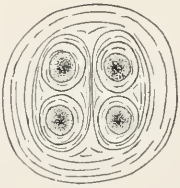
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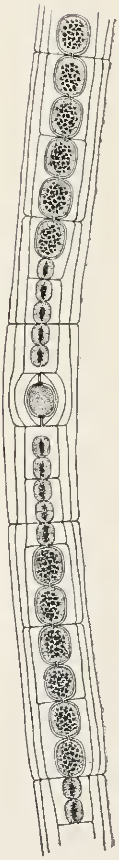
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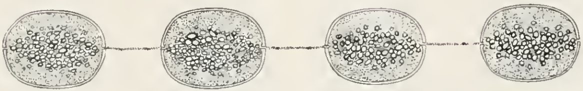
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Phillips on Cyanophyceæ.

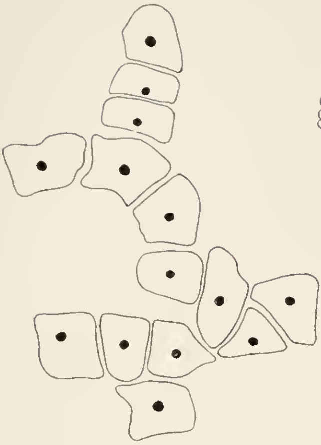
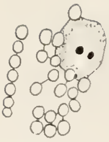


Fig. 1



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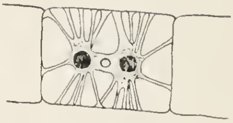

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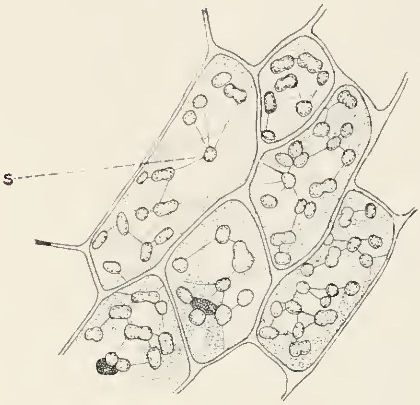

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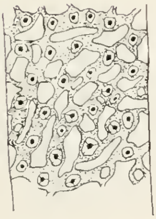

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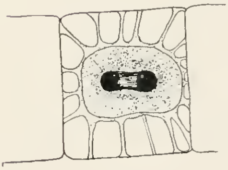

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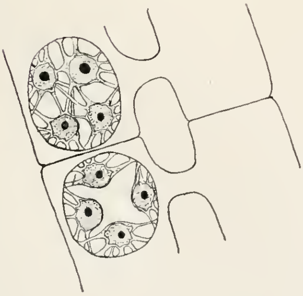

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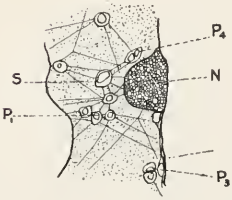

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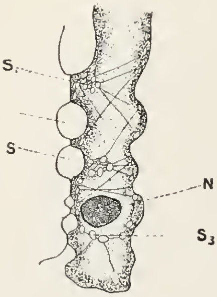
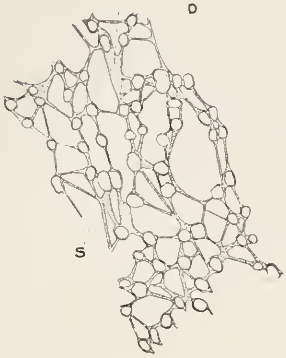


Fig. 9.

WATSON ON PLASTIDS.



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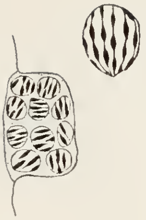

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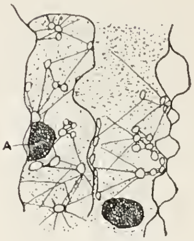

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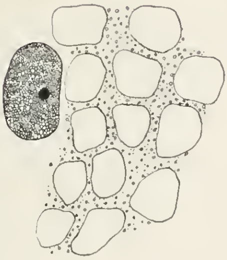

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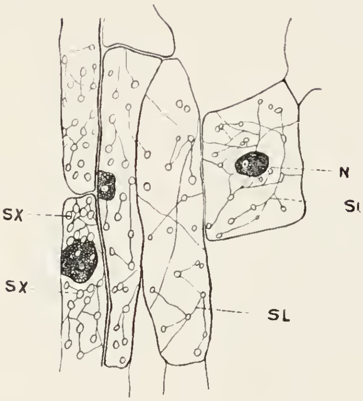

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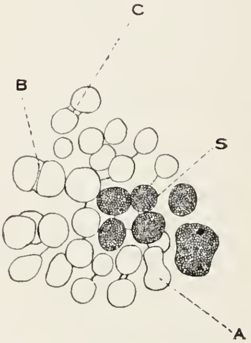

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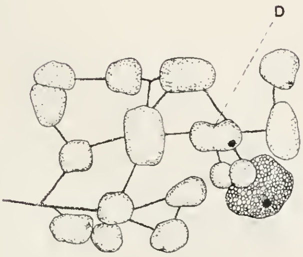

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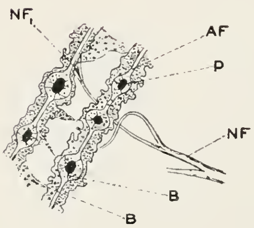
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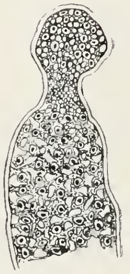
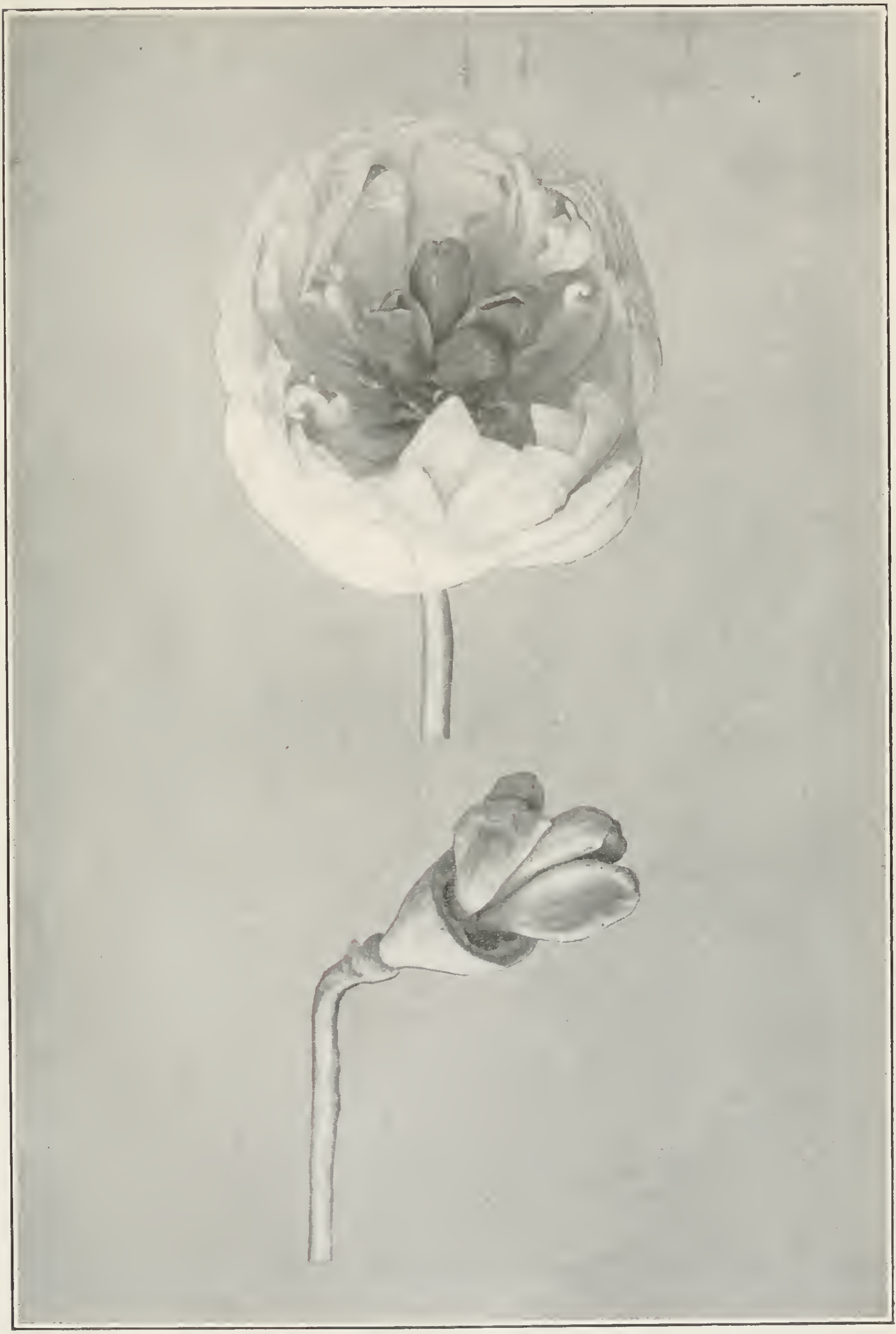


Fig. 17.

WATSON ON PLASTIDS.



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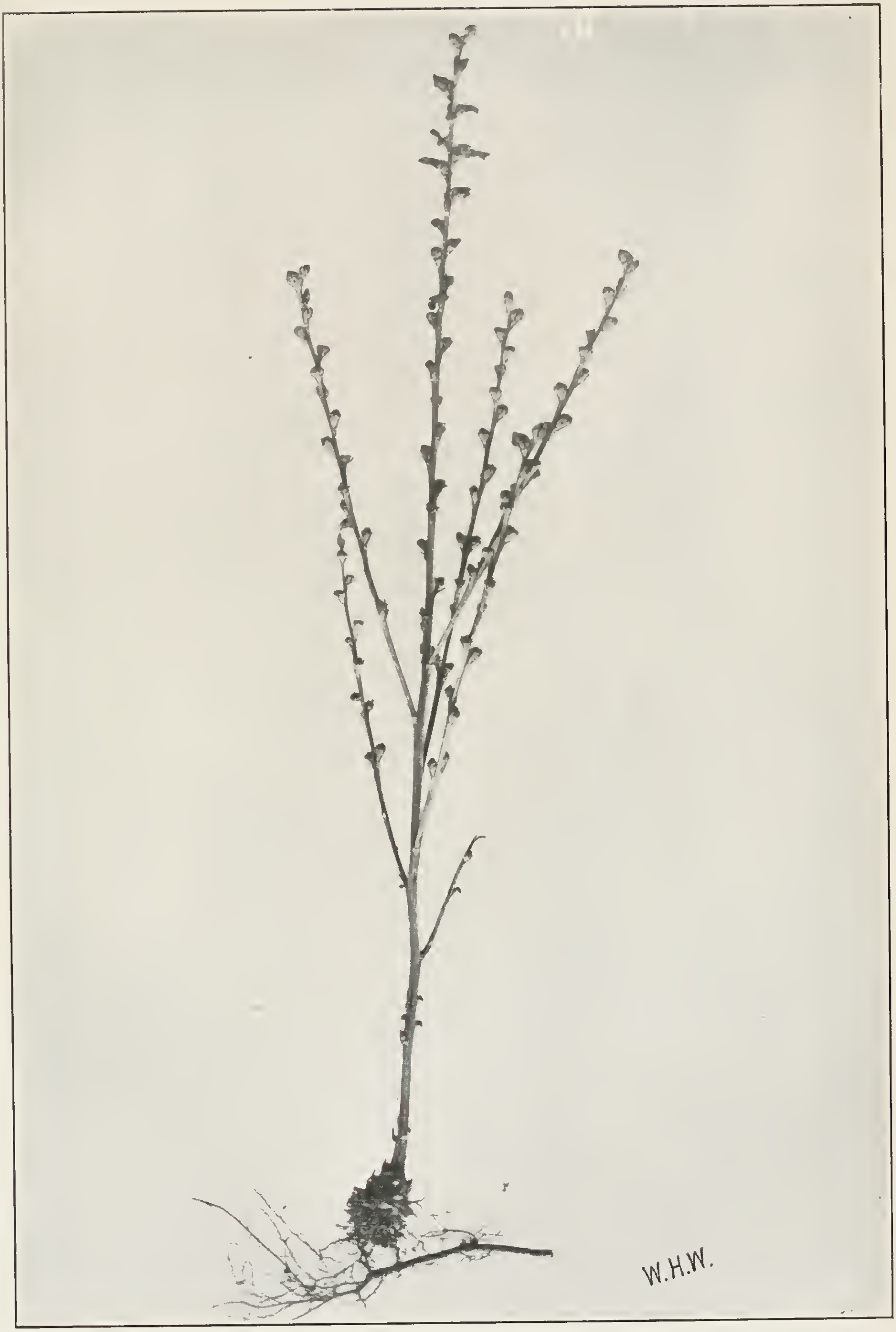
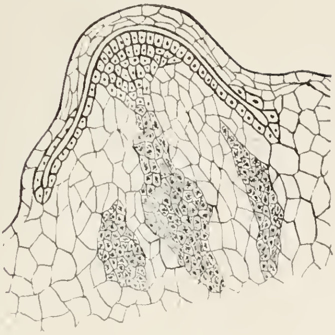


STRUCTURE OF EPIPHEGUS.



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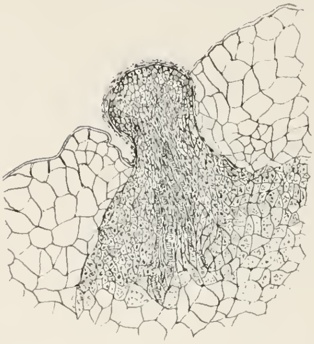

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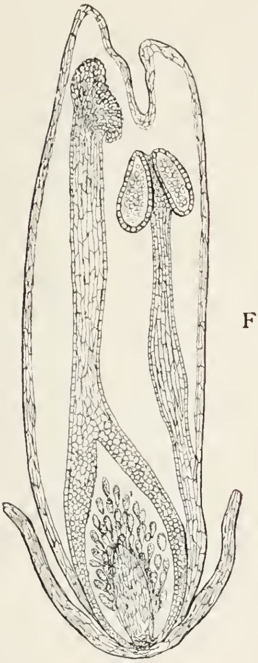

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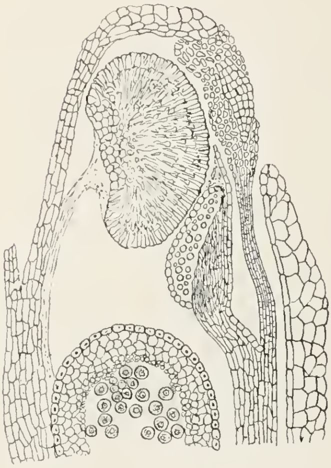

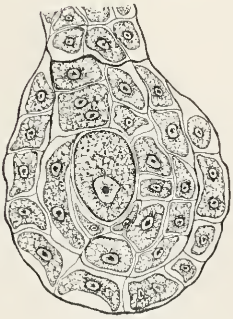

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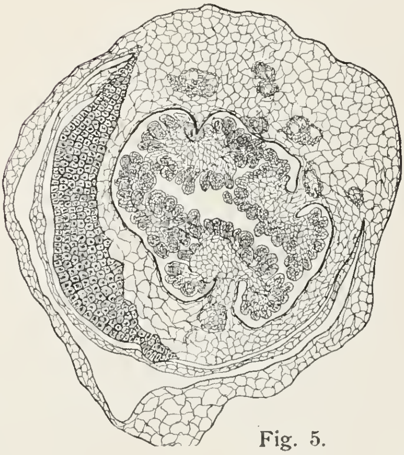

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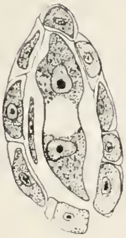
STRUCTURE OF EPIPHEGUS.



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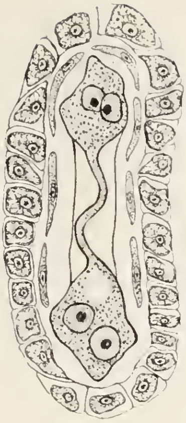

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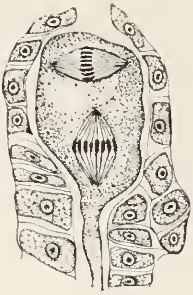

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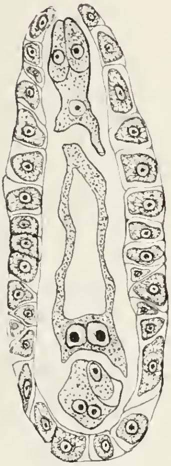

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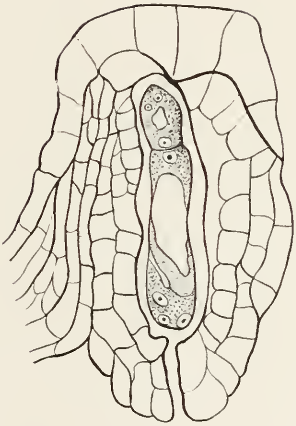

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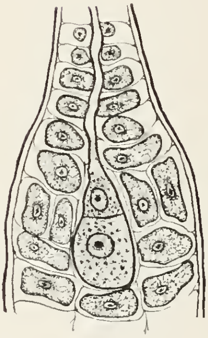

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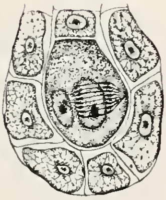

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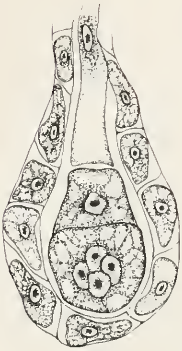

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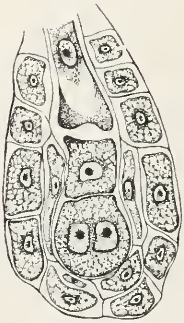
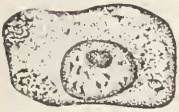


Fig. 15.

STRUCTURE OF EPIPHEGUS.



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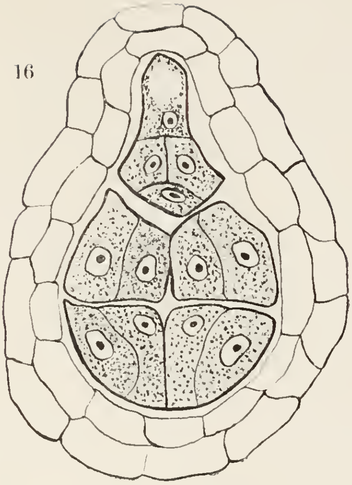

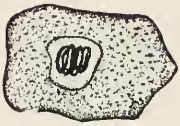

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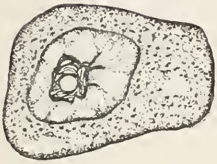

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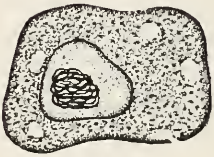

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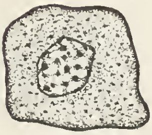

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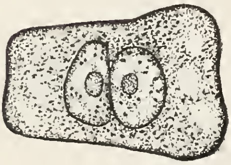

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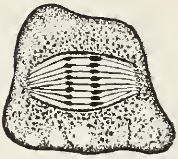

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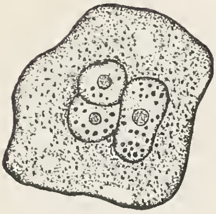

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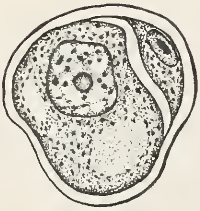

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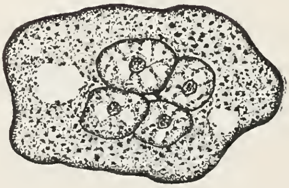

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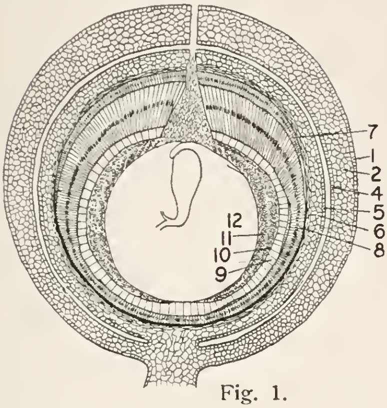
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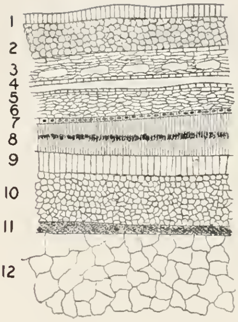
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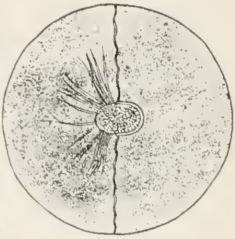

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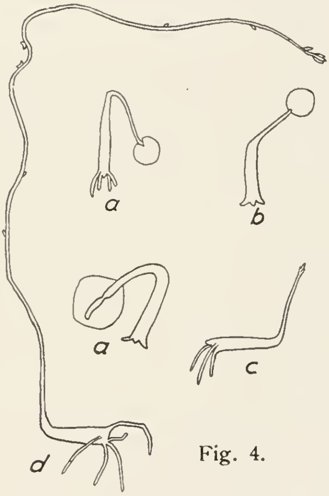

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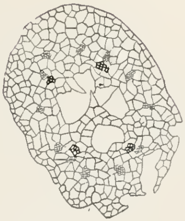

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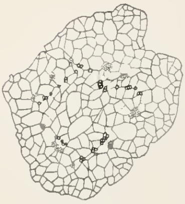

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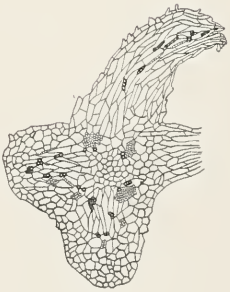
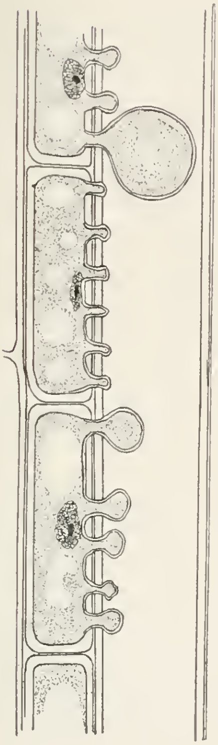


Fig. 7.

BOEWIG ON CASSYTHA.



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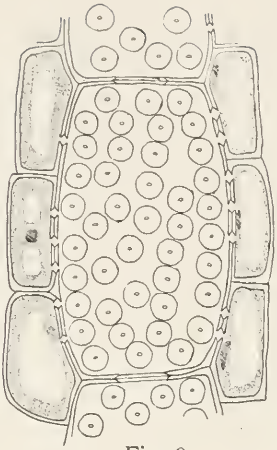

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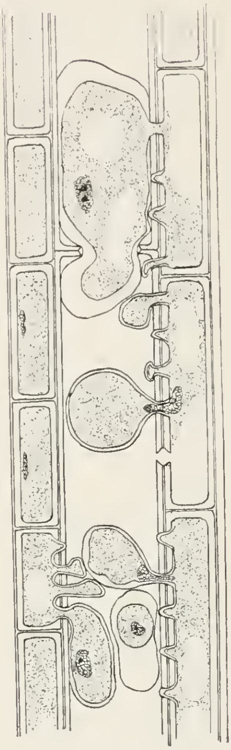

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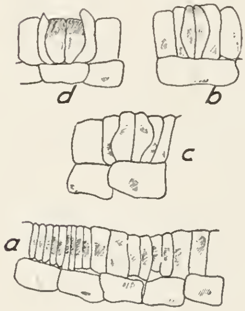

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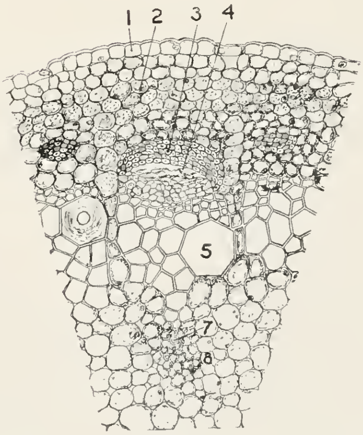

Fig. 8.

Boewig on Cassytha.

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